An investigation of the association between maternal body weight and vitamin D status during pregnancy

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I confirm that the word count of this thesis is less than 100,000 words

To my family

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Summary

The combined effects of maternal obesity and low vitamin D status have adverse implications for maternal and fetal health. The overall aim of this PhD thesis was to investigate the association between maternal body weight and vitamin D status throughout pregnancy. From the systematic review, this thesis reports that few observational studies have assessed vitamin D status across BMI categories. Obese or obese class 2 pregnant women had lower vitamin D status compared to non-obese pregnant women. Maternal vitamin D status was negatively associated with maternal BMI and positively associated with infants vitamin D status. Vitamin D status was lower in infants born to obese mothers than infants born to normal weight mothers. When we examined data from a previous cohort of pregnant women in Northern Ireland (from 2006) who were not taking vitamin D supplements in pregnancy, we were able to examine the true association between BMI and vitamin D status in this unique cohort. Our results demonstrate that pregnant women with obesity had significantly lower vitamin D status than those who were normal weight, this was evident particularly during the winter months. There was a high prevalence of vitamin D insufficiency (<50 nmol/L) among pregnant women living in Northern Ireland in 2006. When we assessed the association between BMI and vitamin D status in early pregnancy in a cohort that were recruited for the purposes of this PhD work, we found that pregnant women with overweight and obesity had significantly lower vitamin D status compared to pregnant women of normal weight in early pregnancy despite 62% of women reporting to take a vitamin D supplement. Maternal BMI was found to be a significant negative predictor of vitamin D status and a high prevalence of vitamin D insufficiency still exists among pregnant women living in Northern Ireland in 2017. This insufficiency was highest among non-supplement users during winter months.

We assessed the effect of supplementation with 10µg vs 20µg vitamin D_3/d throughout pregnancy on vitamin D status of normal weight, overweight and obese pregnant women and on the cord blood of their infants. We report that vitamin D status increased throughout the pregnancy in both treatment groups, with a significantly higher increase observed in the 20µg group. In the 20µg group, maternal and cord vitamin D status reached and maintained sufficiency (≥50 nmol/L) throughout pregnancy, even in those who started pregnancy with an insufficient status. In obese women who started pregnancy with an insufficient vitamin D status, the related cord blood vitamin D status was deficient (<25 nmol/L) in both treatment groups (4). Overall, maternal obesity was negatively associated with maternal and infant vitamin D status and the current recommendation of 10µg/d is inadequate to reach and maintain sufficiency in those who start pregnancy with an insufficient status.

Abbreviations

%	Percentage
ºC	Degrees Celsius
µg	Microgram
25(OH)3D	25-hydroxy vitamin D
1,25(OH)2D	1 ,25-dihydroxyvitamin D
ANOVA	Analysis of variance
BMI	Body mass index
BP	Blood pressure
Ca	Calcium
CC	Case-control study
CLIA	Chemiluminescence immunoassay
cm	Centimetres
CRP	C-reactive protein
CS	Caesarean section
CSS	Cross-sectional study
d	Day
DBP	Diastolic blood pressure
ECLIA	Electrochemoluminescent Immunoassay
EIA	Enzyme immunoassay

ELISA	Enzyme linked immunosorbent assays
FFQ	Food frequency questionnaire
g	Gram
GDM	Gestational diabetes mellitus
HCP	Health care provider
HDL	High-density lipoprotein
HPLC	High performance liquid chromatography
IDS	Immunodiagnostic system
IU	International units
kg	Kilograms
L	Litre
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDL	Low-density lipoprotein
mg	Milligrams
ml	Millilitres
mmHg	Millimetre of mercury
mmol	Millimoles
mths	Months
n	Number of subjects

NICHE	Nutrition Innovation Centre for Food and Health
ORECNI	Office of Research Ethics Committees for Northern Ireland
P	Probability (level of significance)
r	Correlation value
RIA	Radioimmunoassays
SACN	Scientific Advisory Committee on Nutrition
SD	Standard deviation
SPSS	Statistical analysis package for social sciences
SBP	Systolic blood pressure
UK	United Kingdom
USA	Unites States of America
WHO	World Health Organisation
WHSCT	Western Health and Social Care Trust
wk	Week
yrs	Years
FSA	Food Standard Agency
RCT	Randomised Control Trial
UVB	Ultraviolet (UV) B

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Statement of collaboration

This research was conducted in the Nutrition Innovation Centre for Food and Health (NICHE), Ulster University Coleraine.

The following were carried out by the author of this thesis: participant recruitment, study execution, blood processing, laboratory analysis of calcium and albumin concentrations, collection of data, statistical analysis. Mr Neil Dennison assisted with the laboratory analysis.

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Chapter 1:

Introduction and thesis outline

Maternal obesity

Overweight and obesity are increasing in women of childbearing age (National Obesity Observatory, 2012), with approximately 60% of women of childbearing age (16-44 years) being obese in England and Northern Ireland (Statistics on Obesity, England 2017; Health survey Northern Ireland 2016/17). Obesity is recognised as a major public health concern during pregnancy (Catalano, 2007). The UK has the highest level of maternal obesity in Europe (Poston *et al.,* 2016) with 20% of women classified as obese at the first antenatal appointment (Maternity Services, Monthly Statistics, 2017); in Northern Ireland this figure is estimated at 17% (Scott-Pillai *et al.,* 2013). Body mass index (BMI), defined as weight in kilograms divided by height in meters squared (kg/m^2) is used to categorise individuals into; underweight BMI < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, obese class I 30.0–34.9 kg/m^2, obese class II 35.0–39.9 kg/m^2 and obese class III ≥ 40 kg/m^2 (WHO, 2000).

Obesity related maternal and infant health risks

Maternal obesity significantly contributes to adverse pregnancy and birth outcomes (Chu *et al.,* 2009) and is associated with short and long-term metabolic dysfunction in the mother and her infant (Catalano *et al.,* 2009).

Maternal risks

Maternal obesity is associated with an increased risk of hypertensive disorders of pregnancy, including preeclampsia and hypertension (Salihu *et al.,* 2012). Pregnant women who are overweight or obese are shown to have an increased risk of preeclampsia of approximately 2 to 3 fold when compared to women of normal weight

(Bodnar *et al.,* 2005). This association between obesity and the risk of preeclampsia and hypertension has been reported globally (Mahomed *et al.,* 1998; Hauger *et al.,* 2008; Persson *et al.,* 2016; El-Chaar *et al.,* 2013; Denison *et al.,* 2014) and a study by Jeyabalan, (2013) estimated that 30% of preeclampsia risk is attributable to obesity. Ducarme *et al.* (2007) supports this concept and reported that weight loss prior to pregnancy significantly reduced the risk of preeclampsia. The aetiology of preeclampsia and gestational hypertension include factors such as insulin resistance, genetic predisposition, immunology, poor diet and decreased physical activity (Salihu *et al.,* 2012). Furthermore, maternal obesity is associated with an increased risk of gestational diabetes mellitus (GDM), which has been defined as either a fasting plasma glucose level of ≥5.6 mmol/litre or a 2-hour plasma glucose level of ≥7.8 mmol/litre during pregnancy (NICE, 2015). Insulin resistance is increased throughout pregnancy by action of the placenta and mothers with obesity have a higher insulin resistance (lower insulin sensitivity) than normal weight mothers, which contributes to hyperglycaemia and GDM (Korkmaz *et al.,* 2016). Women who are overweight or obese have an increased relative risk of developing GDM by 1.3-3.8 times compared with women who are normal weight (Kim *et al.,* 2013). It is also well documented that women with GDM are at a higher risk for GDM in future pregnancies (Getahun *et al.,* 2004), as well as being at an increased risk of developing diabetes later in life (Bellamy *et al.,* 2009). Preterm delivery is also considered to be one of the consequences of maternal obesity and is defined as a delivery of an infant before 37 gestational weeks (Simmons *et al.,* 2010). Women with obesity classes 2 and 3 have an increased risk of preterm delivery between 32-36 weeks and <32 weeks, respectively (McDonald *et al.,* 2010; Torloni *et al.,* 2009). Two retrospective studies of pregnant women in Florida and Finland observed an increased risk of preterm delivery (<28 weeks) in obese

women with BMI ≥30 kg/m^2 (Salihu *et al.*, 2010; Raisanen *et al.*, 2013). However, associations between preterm delivery and overweight (BMI 25-29.9 kgm^2) and obesity class 1 (BMI 30≤35 kg/m^2) are less consistent (Nohr *et al.*, 2007; Torloni *et al.*, 2009). The mechanism between preterm delivery and maternal obesity is unknown but is believed to be associated with alterations in immune function (Goldenberg *et al.*, 2008). Maternal obesity is also a major contributing factor to the increased global rates of caesarean section (CS), especially in developed countries (Barry *et al.*, 2009). Previous meta-analyses have shown a 2-fold increase in CS in obese women compared to normal weight women (Heslehurst *et al.*, 2008; Poobalan *et al.*, 2009) and a study by O'Dwyer *et al.* (2011) reported that maternal obesity in European women is associated with an increase in emergency CS in primigravidas and an increase in elective CS in multigravidas, compared with women who are normal weight.

Infant risks

Maternal obesity contributes to short and long-term adverse health effects in the infant. Infants born to mothers with obesity have an increased risk of abnormal growth such as macrosomia, (defined as birth weight higher than 4000g or 4500g), as well as an increased risk of being large for gestational age (LGA), (defined as birth weight higher than the 90th centile for gestational age) (Catalano and Shankar, 2017). Studies have reported that mothers with obesity have a 2-fold higher risk of giving birth to an LGA infant compared to mothers of normal weight (Gaudet *et al.*, 2014). These risk factors could be related to insulin resistance as infants of mothers with obesity have higher insulin resistance than those of normal weight mothers, with a positive relationship shown between maternal pre-pregnancy BMI and fetal insulin resistance (Catalano *et*

al., 2009). Furthermore, children born to mothers with obesity are at a higher risk of long-term adverse health outcomes than children born to mothers of normal weight, such as cardiovascular disease (Reynolds *et al.,* 2013), type-2 diabetes (Morgan *et al.,* 2010) and a 3-fold increased risk of childhood obesity (Yu *et al.,* 2013; Taveras *et al.,* 2009).

Vitamin D metabolism in pregnancy

Vitamin D is a fat-soluble vitamin involved in calcium and phosphate homeostasis and is essential for the maintenance of bone health. Severe vitamin D deficiency causes rickets in children and osteomalacia in adults (Bouvard *et al.,* 2011; Christakos *et al.,* 2011). There are two forms of vitamin D; D_3 (cholecalciferol) which is obtained from animal sources or produced from 7-dehydrocholesterol in the skin following exposure to ultraviolet B irradiation from sunlight, and D_2 (ergocalciferol) which is obtained from plant sources. Vitamin D undergoes hydroxylation in the liver by the hydoxylase enzyme 25-hydroxylase (CYP27A1) to form 25 hydroxyvitamin D (25(OH)D, the status marker of the vitamin) and a further hydroxylation in the kidneys and other organs by the enzyme 1-alpha hydroxylase (CYP27B1) to produce the biological active form 1,25 dihydroxyvitamin D (1,25(OH)2D). The active form is responsible for increasing calcium absorption/resorption from the small intestine, increasing urine reabsorption/excretion, regulating parathyroid hormone (PTH) and increasing bone mineralisation. Adequate vitamin D is required to maintain homeostasis and calcium supply to all tissues and organs in the body, particularly the heart, bone, skeletal muscle and brain (Wagner *et al.,* 2012).

Vitamin D metabolism is altered during pregnancy to meet the physiological demands of the mother and fetus. The conversion of 25(OH)D to $1,25(OH)_2D$ is increased approximately 2-fold throughout pregnancy (Hollis *et al.,* 2011). During pregnancy, 25(OH)D is transferred across the placenta and it has been shown that fetal cord blood concentrations of 25(OH)D correlate with maternal 25(OH)D status (Shin *et al.,* 2010). Whilst, $1,25(OH)_2D$ does not readily cross the placenta (Shin *et al.,* 2010; Kaludjerovic *et al.,* 2010), fetal kidneys and placental tissue express CYP27B1 to convert 25(OH)D to $1,25(OH)_2D$ within fetal circulation (Adams and Hewison, 2012) (Figure 1). In the infant, cord blood concentrations of 25(OH)D and $1,25(OH)_2D$ positively correlate (Walker *et al.,* 2011, Eichholzer *et al.,* 2013). Previously it was believed that concentrations of calcium for adequate maternal and fetal skeletal requirements were maintained owing to an increase in circulating concentrations of $1,25(OH)_2D$ during pregnancy (Hollis *et al.,* 2011). However, the increase in $1,25(OH)_2D$ concentrations in early pregnancy is not thought to be linked to calcium homeostasis as calcium requirements only significantly increase in the third trimester and during lactation (Carneiro *et al.,* 2010). Approximately 25-30g of calcium is transferred from the mother to the fetal skeleton during the last trimester (Kaushal and Magon, 2013). The rate of conversion to $1,25(OH)_2D$ in the mother and fetus is dependent on 25(OH)D substrate availability and increased CYP27B1 activity in the placenta and decidua and is largely independent of calcium homeostasis (Hollis *et al.,* 2010; Liu and Hewison, 2011; Adams and Hewison, 2012; Hewison *et al.,* 2004). It has been reported that pregnant women who develop preeclampsia have abnormal expression of CYP27B1, presenting a potential role for $1,25(OH)_2D_3$ as a regulator of placentation which is suggestive of a protective mechanism of vitamin D on fetal-placental development (Kaushal and Magon 2013). Furthermore, it has been reported

by Bakacak *et al.* (2015) that vitamin D is involved in regulating genes in early placental development. More recently, vitamin D has also been found to play a role in reproduction as well as implantation of the embryo (Heyden and Wimalawans, 2018).

Current recommendations for vitamin D during pregnancy:

The main source of vitamin D is from sun exposure accounting for approximately 90%, while the remaining 10% comes from a small range of foods such as liver, egg yolks, fatty fish and mushrooms, fortified foods and dietary supplements (Laird *et al.*, 2010). Cutaneous synthesis of vitamin D is affected by many factors including latitude, season, sunscreen use, pollution, weather, age and skin colour. In the UK, vitamin D deficiency (<25 nmol/L) is three times more common in winter and spring compared to summer and autumn (Hypponen and Power, 2007). Hill *et al.* (2004) reports that latitudes between 51-55°N in Ireland contribute greatly to vitamin D deficiency leading to a reliance on foods containing vitamin D during winter and autumn, and stores built up from sun exposure in the previous summer. In the UK the mean daily intake of vitamin D from food sources for women aged 19-64 years ranges from 2.2 to 2.8µg/d (National Diet and Nutrition Survey NDNS,2008/09 to 2011/12, (Bates, 2014), well below the currently recommended intakes of 10µg/d for all adults including pregnant women (SACN, 2016). Compounding these low intakes, pregnant women are advised to reduce intake of some of the naturally containing vitamin D rich foods such as liver and fish owing to safety issues and risk of bacterial exposure (National Health Service, 2012; Food Standards Agency, 2008).

Prevalence of low vitamin D status during pregnancy:

Vitamin D deficiency and insufficiency among pregnant women have been reported worldwide (Dawodu *et al.,* 2012). Studies have reported vitamin D insufficiency during pregnancy in European countries (<50 nmol/L) such as Spain (27%) (Rodríguez-Dehli *et al.,* 2015) and Poland (24%) (Bartoszewicz *et al.,* 2013). Furthermore, in North India (92%) (Singla *et al.*, 2015), and Qatar (48%) (Bener *et al.,* 2013). Studies have reported vitamin D deficiency (<25 nmol/L) in various populations including Riyadh (68%) (Al-Ajlan *et al.,* 2015) and almost 60% in Tokyo (Shiraishi *et al.,* 2014). In the UK, the prevalence of vitamin D deficiency (<25nmol/L) among pregnant women living in London was reported to be 47%, 64%, 58% and 13% in Asian, Middle Eastern, Black and Caucasian women respectively (Yu *et al.,* 2009). Furthermore, approximately 23% of healthy pregnant women living in Glasgow were found to be vitamin D deficient (Makgoba *et al.,* 2011). Over 90% of pregnant women living in Northern Ireland were vitamin D insufficient and insufficiency was also found in women taking vitamin supplements (Holmes *et al.,* 2009).

Consequences of low vitamin D status during pregnancy for mother and infant:

Recently, a number of studies have reported adverse health outcomes for the mother and the infant with vitamin D deficiency during pregnancy (Karras *et al.,* 2013) and these outcomes mirror those associated with obesity.

Maternal risks

Low vitamin D status in early pregnancy has been observed in women who developed pre-eclampsia later in pregnancy and a 50nmol/L decline in vitamin D doubled the risk of preeclampsia (Bodnar *et al.,* 2007). However, these associations have not been

supported by randomised controlled trials which have reported no significant effects of vitamin D supplementation (110µg/d) on incidence of preeclampsia when compared to a lower dose (10µg/d) of vitamin D from early in pregnancy (10-18 weeks) up to the third trimester (32-38 weeks) (Mirzakhani *et al.,* 2016). However, it was noted that having a 25(OH)D concentration of >75 nmol/L throughout pregnancy was associated with a lower risk of preeclampsia (Mirzakhani *et al.,* 2016). A meta-analysis of 6 studies suggested an association between higher vitamin D status and a reduced risk of pre-eclampsia, and 4 randomised supplementation studies were suggestive of a protective association (Hyppönen *et al.*, 2013).

In addition, meta-analyses have shown that low vitamin D status is associated with increased incidence of GDM (Aghajafari *et al.,* 2013). In a more recent observational study of Chinese pregnant women, vitamin D status at the first prenatal visit was significantly lower in women who developed GDM compared with those who did not (Xu *et al.,* 2017). Furthermore, supplementation studies have supported the associations between vitamin D and GDM. In a randomized, double-blind, placebo-controlled trial, 90 pregnant women who had at least one risk factor for GDM were randomised to receive either 125µg/d of vitamin D/d or placebo until 26 weeks gestation, and it was reported that the incidence of GDM was significantly lower in the intervention group compared to the control group (Shahgheibi *et al.,* 2016). Furthermore, a randomised clinical trial using a dose of 1250µg vitamin D every 2 weeks from 12 weeks of pregnancy until delivery was found to significantly improve insulin resistance compared to other groups who received either 5µg vitamin D daily or 1250µg vitamin D monthly (Soheilykhah *et al.*, 2013). In contrast, a recent study by Tehrani *et al.* (2017) found that vitamin D supplementation of 125µg every 2 weeks

for 10 weeks among those who had low vitamin D status (<25 nmol/L) at the start of the study had no effect on incidence of GDM during pregnancy.

Vitamin D has also been reported to play a role in obstetric outcomes and low maternal vitamin D status (<37.5 nmol/L) at delivery has been shown to be associated with an increased risk of CS (Merewood *et al.,* 2009). Furthermore, maternal vitamin D deficiency (<30 nmol/L) was reported to result in a 2-fold increased risk of caesarean due to prolonged labour in a large cohort of pregnant women in the United States (Theresa *et al.,* 2012). Some studies have also reported associations between maternal vitamin D status in early pregnancy and type of delivery however, within a UK study no significant differences in mode of delivery according to maternal vitamin D status in first trimester were observed (Savvidou *et al.,* 2012).

Infant risks

Low maternal vitamin D status has also been linked with a number of adverse outcomes in the offspring. Maternal vitamin D deficiency may impair fetal growth and result in adverse outcomes including low neonatal birth weight. A recent cross-sectional study reported that low birth weight neonates (<2500g) were significantly more likely to be born to mothers with low vitamin D status compared to normal birth weight neonates (>2500g) who were more likely to be born to mothers with higher vitamin D status (Khalessi *et al.,* 2015). A positive correlation between maternal vitamin D status and neonatal birth weight has also been reported in a Chinese cohort (Chen *et al.,* 2015) albeit not all studies have found these associations (Morley *et al.,* 2006).

In the long term, children born to mothers who had a low vitamin D status during pregnancy are reported to be more likely to suffer from wheezing and asthma

(Camargo *et al.,* 2007; Erkkola *et al.,* 2009), type 1 diabetes mellitus and insulin resistance (Hypponen *et al.,* 2001; Fronczak *et al.,* 2003) and multiple sclerosis (Mirzaei *et al.,* 2011). Furthermore, maternal vitamin D status also contributes to skeletal development programming (Sayers *et al.,* 2009) and body composition in children (Pasco *et al.,* 2008) by influencing the interaction between osteoblasts and adipocytes. Low maternal vitamin D status during late pregnancy is associated with reduced whole-body bone mineral content, bone area, and areal bone mineral density in children at 9 years old (Javaid *et al.,* 2006). Therefore, it is essential to maintain optimal vitamin D status during pregnancy to prevent vitamin D deficiency in the fetus and subsequent adverse health outcomes (Wagner *et al.,* 2012, Hollis *et al.,* 2004).

Effect of vitamin D supplementation during pregnancy:

The effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes using randomised controlled trials (RCT) have been examined in 5 systematic reviews (De-Regil *et al.,* 2012& 2016, Harvey *et al.,* 2014, Perez-Lopez *et al.,* 2015, Thorne-Lyman and Fawzi 2012) and consistently reported that vitamin D supplementation in a single or continued dose during pregnancy significantly increased maternal vitamin D status at term. A review by Harvey *et al.* (2014) assessed observational and RCT studies and concluded that whilst there was modest evidence to support a relationship between maternal vitamin D status and neonatal birth weight, bone mass and calcium status, these findings are insufficient to support definite clinical recommendations for vitamin D supplementation during pregnancy. A meta-analysis by Thorne-Lyman and Fawzi (2012) which assessed observational and RCTs reported protective effects of vitamin D supplementation on low birthweight and non-

significant but suggestive effects of vitamin D supplementation on small for gestational age and no effect on preterm delivery. A more recent meta-analysis by Perez-Lopez *et al.* (2015) which included 13 RCTs found that vitamin D supplementation during pregnancy was associated with increased vitamin D status, birth weight and birth length, but was not associated with other maternal and neonatal outcomes.

Impact of obesity on vitamin D status for mother and infant

Research in recent years has reported an inverse association between obesity and vitamin D status in many populations (Perez-Lopez *et al.,* 2011, Bartoszewicz *et al.,* 2013). It is thought that this inverse association may be due to decreased sun exposure as obese individual have less outdoor activity and might use more clothing coverage than those who are normal weight (Compston *et al.,* 1981; Vanlint, 2013). Another potential mechanism is increased sequestration in adipose tissue as vitamin D is a fat soluble vitamin (Wortsman *et al.,* 2000). Studies in which obese and normal weight individuals with similar baseline vitamin D status were exposed to UVB irradiation for 24 h reported a 57 % lower vitamin D status in obese individuals post-intervention compared to the normal weight individuals, highlighting the differences in vitamin D synthesis according to weight status (Wortsman *et al.,* 2000). Moreover, it has been proposed by Drinic *et al.* (2012) that volumetric dilution may also result in low circulating concentrations of vitamin D in obese individuals.

A number of studies have been conducted in pregnant cohorts and reported that obese or severely obese pregnant women had significantly lower vitamin D status compared to non-obese pregnant women (Andersen *et al.,* 2013, Bodnar *et al.,* 2007, Perez-

Lopez *et al.,* 2011, Perampalam *et al.,* 2011, Bartoszewicz *et al.*, 2013, McAree *et al.,* 2013, van den Berg *et al.,* 2013, Karlsson *et al.,* 2015). In a cohort of pregnant women from Denmark, it was observed that a 5 unit increase in BMI during both winter and summer correlated to a lower 25(OH)D of 4.2 nmol/L and 2.85 nmol/L respectively (Andersen *et al.,* 2013). However, in a cross-sectional study of pregnant women by Pena *et al.* (2015) the authors noted that vitamin D status was not significantly influenced by obesity, despite the obese pregnant women and their neonates having significantly higher incidence of vitamin D deficiency, albeit this was not significantly different when compared to non-obese pregnant women and their infants.

The fetus is completely reliant on the maternal vitamin D status and supply (Hollis & Pittard, 1984) and whilst neonatal vitamin D status is highly correlated with maternal vitamin D status (Dror *et al.,* 2011, El Rifai *et al.,* 2014, Godang *et al.,* 2014), obesity significantly weakens this correlation (Pena *et al.,* 2015). Neonates of obese mothers had significant lower vitamin D in cord blood than neonates of normal weight mothers and the author suggested that obese women and their neonates are at high risk of vitamin D deficiency even when the mothers regularly use prenatal vitamins containing vitamin D (Bodnar *et al.,* 2007). Jami *et al.* (2013) have suggested that a higher dose of vitamin D supplementation is required for obese pregnant women than normal weight pregnant women. It has been observed that normal weight pregnant women transfer more vitamin D across the placenta to the fetus than obese pregnant women, even when maternal 25(OH)D concentrations did not differ. Indeed, vitamin D deficiency during pregnancy is recognized as a serious concern for pregnant women especially if they are obese and have dark skin (McAree *et al.,* 2013, Dijkstra *et al.,* 2007, Finer *et al.,* 2012, Bodnar *et al.,* 2007).

Independent of each other obesity and low vitamin D status during pregnancy lead to adverse consequences for the mother and their infants. However, the combined effect of both obesity and low vitamin D status among healthy women during pregnancy is poorly documented and the incidence of both during pregnancy may contribute to increased adverse health outcomes for the mother and their infants. Vitamin D status during pregnancy has been previously investigated but few studies have considered the potential implications of obesity. In addition, whilst the effect of vitamin D supplementation during pregnancy on vitamin D status has been assessed, maternal body weight has not been taken into account. Furthermore, the effect of vitamin D supplementation during pregnancy among normal weight, overweight and obese pregnant women and their infant has not yet been investigated.

The influence of maternal obesity on vitamin D status is an important public health consideration with potential short and long-term adverse health consequences for both the mother and her infant. Therefore, the overall aim of this thesis is to assess the association between maternal body weight and vitamin D status throughout pregnancy.

This aim has been addressed through the completion of several chapters as follows:

1- The association between maternal obesity and vitamin D status of mothers and infants: a systematic review (Chapter 2)

Aim: To explore current knowledge on the association between maternal obesity and vitamin D status of the mother and their infants cord blood through a systematic review

2- The association of overweight and obesity on vitamin D status during pregnancy using data from the FASSTT Study (Chapter 3)

Aim: To investigate the association between BMI and maternal vitamin D status and to investigate for associations between vitamin D status and neonatal birth outcomes.

3- The association between maternal body weight and vitamin D status in early pregnancy (Chapter 4)

Aim: To assess and compare maternal vitamin D status between normal weight, overweight and obese pregnant women in early pregnancy

4- The effect of supplementation of 10µg vs 20µg vitamin D_3/d on vitamin D status in normal weight, overweight and obese pregnant women (Chapter 5)

Aim: To assess the effect of supplementation of 10µg vs 20µg vitamin D_3/d throughout pregnancy on vitamin D status of normal weight, overweight and obese pregnant women and on the cord blood of their infants.

5- Discussion (Chapter 6)

Aim: To discuss and interpret the overall association between maternal obesity and vitamin D status and the association between maternal and neonatal health and to identify future work in the area of maternal obesity and vitamin D in pregnancy.

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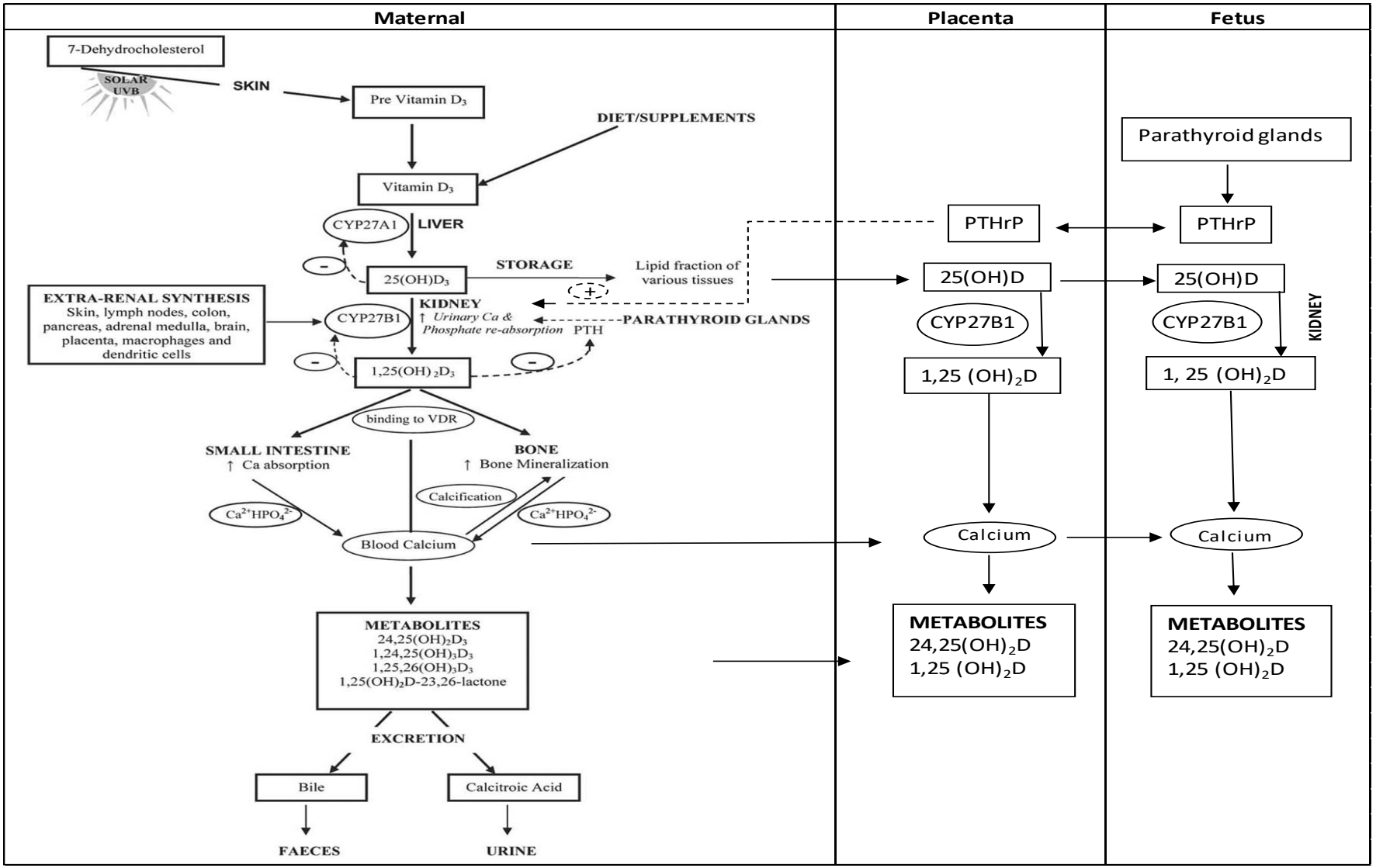
Maternal
Placenta
Fetus
7-Dehydrocholesterol
SOLAR UVB
SKIN
Pre Vitamin D3
DIET/SUPPLEMENTS
Vitamin D3
CYP27A1
LIVER
25(OH)D3
STORAGE
Lipid fraction of various tissues
EXTRA-RENAL SYNTHESIS
Skin, lymph nodes, colon, pancreas, adrenal medulla, brain, placenta, macrophages and dendritic cells
CYP27B1
KIDNEY
↑ Urinary Ca & Phosphate re-absorption
PARATHYROID GLANDS
PTH
1,25(OH)2D3
binding to VDR
SMALL INTESTINE
↑ Ca absorption
BONE
↑ Bone Mineralization
Calcification
Ca2+HPO42-
Blood Calcium
METABOLITES
24,25(OH)2D3
1,24,25(OH)3D3
1,25,26(OH)3D3
1,25(OH)2D-23,26-lactone
EXCRETION
Bile
Calcitroic Acid
FAECES
URINE
PTHrP
25(OH)D
CYP27B1
1,25 (OH)2D
Calcium
METABOLITES
24,25(OH)2D
1,25 (OH)2D
Parathyroid glands
PTHrP
25(OH)D
CYP27B1
KIDNEY
1, 25 (OH)2D
Calcium
METABOLITES
24,25(OH)2D
1,25 (OH)2D

Figure1. Diagram of vitamin D metabolism in pregnancy (adapted from Barnes et al., 2006).

During pregnancy, maternal vitamin D obtained from sun exposure or through diet is hydroxylated in the liver by the hydoxylase enzyme 25-hydroxylase (CYP27A1) to form 25 hydroxyvitamin D (25(OH)D, the status marker of the vitamin) before undergoing a further hydroxylation step in the kidneys by the enzyme 1-alpha hydroxylase (CYP27B1) in response to parathyroid hormone (PTH) to form the biologically active form 1,25 dihydroxyvitamin D ($1,25(OH)_2D$). $1,25(OH)_2D$ binds to vitamin D receptors (VDR) on target organs (small intestine, bone, ,muscle, placenta, immune system) to and produces the appropriate biological response increase calcium absorption. Any unused vitamin D is converted to various metabolites and excreted in the faeces or urine. During pregnancy, maternal 25(OH)D crosses the placenta to the fetus and is the only source of vitamin D. Whilst $1,25(OH)_2D$ does not as readily cross the placenta, the placental tissue and fetal kidneys express CYP27B1 to enable conversion of 25(OH)D to 1,25 $(OH)_2D$ to meet fetal physiological demands to maintain calcium homeostasis for fetal bone development. PTH-related peptide (PTHrP) produced in fetal parathyroid glands and placental tissues and increases placental synthesis of $1,25(OH)_2D$. PTHrP could mediate the increased $1,25(OH)_2D$ and help regulate calcium and PTH levels during pregnancy.

Chapter 2:

The association between maternal obesity and vitamin D status of mothers and infants: a systematic review

Abstract

Maternal obesity is becoming increasingly common and significantly contributes to adverse pregnancy and birth outcomes. Vitamin D insufficiency is another established risk factor in pregnancy and is linked with health consequences similar to those associated with obesity.

The association between maternal obesity and low vitamin D status is an important public health consideration. The aim of this systematic review is to explore current knowledge on the association between maternal obesity and vitamin D status of mothers and their infants cord blood.

Literature searches were conducted using Ovid MEDLINE, PubMed, Web of science and Cochrane library databases. Papers published from 1 January 1980 to 17 June 2018 following PRISMA guidelines were included in the search. Inclusion criteria were: articles reporting quantitative body composition measures or categories of BMI and vitamin D status (as 25(OH)D concentrations), data from any study design, published in the English language and including data on healthy pregnant women and their infants. Two randomised clinical trials and 58 observational studies were included in this review.

As evidenced by our findings, few observational studies have assessed vitamin D status across BMI categories. Obese or obese class 2 pregnant women had lower vitamin D status compared to non-obese pregnant women. Maternal vitamin D status was positively associated with infants vitamin D status but was negatively associated with maternal BMI. Vitamin D status was lower in infants born to obese mothers than infants born to normal weight mothers; this difference was related to factors such as maternal vitamin D status, maternal obesity, maternal age and infant adiposity.

Maternal obesity was negatively associated with maternal and infant vitamin D status. Advice on vitamin D supplementation during pregnancy may need to be based on pre-pregnancy BMI.

Introduction

Maternal obesity (defined as body mass index (BMI) ≥30 kg/m^2) is becoming increasingly common and significantly contributes to adverse pregnancy and birth outcomes (Chu *et al.,* 2009). The prevalence of maternal overweight and obesity across Europe is estimated at 30-37% (European perinatal health report, 2010). In the UK, 20% of women are classified as obese at their first antenatal appointment (Maternity Services Monthly Statistics, 2017). Obesity-related consequences during pregnancy include increased risk of gestational diabetes (GDM), preeclampsia, hypertension, caesarean section (CS), preterm delivery and low birth weight (Scott-Pillai *et al.,* 2013).

Vitamin D insufficiency during pregnancy has been associated with adverse health consequences similar to those of obesity (McAree, 2013), and there is a high prevalence of low vitamin D status in many populations globally (Dawodu *et al.,* 2012). During pregnancy, there are increased calcium demands for fetal bone growth and development (Ponsonby *et al.,* 2010) and the fetus relies solely on the mother for its supply of vitamin D to meet these demands. Furthermore, obesity is a recognised risk factor for hypovitaminosis D in the general population (Vimaleswaran *et al.,* 2013) as well as during pregnancy (Bodnar *et al.,* 2007; Karlsson *et al.,* 2015). Indeed, Andersen *et al.,* (2013), reported that a 5 kg/m^2 increase in BMI was associated with a decrease in vitamin D status of 3.7 nmol/L in pregnant women.

Although limited in number, some epidemiological studies have shown that obese pregnant women have a significantly lower vitamin D status than non-obese women (Bodnar *et al.,* 2007; Karlsson *et al.,* 2015; McAree *et al.,* 2013), and it has been shown that obese pregnant women transfer less vitamin D to the infant than normal-weight

pregnant women even when maternal vitamin D status is the same (Josefson *et al.*, 2013). In addition, Karlsson *et al.*, (2014) reported that, despite having a higher dietary vitamin D intake and similar supplement use, obese pregnant women had lower vitamin D status in the first trimester compared with normal weight women. Several potential mechanisms explaining low vitamin D status in obese pregnant women have been postulated including increased sequestration in adipose tissue and owing to volumetric dilution (Parikh *et al.*, 2004; Gallagher *et al.*, 2013).

The association between maternal obesity and low vitamin D status in the mother and their infants is an important public health consideration but one that is poorly understood. The aim of this systematic review is to explore current knowledge on the association between maternal obesity and vitamin D status of the mother and their infants cord blood.

Methods

Search strategy

A systematic search strategy was developed and electronic literature searches were conducted using Ovid MEDLINE (www.gateway.Ovid.com), PubMed (www.PubMed.org), Web of science (www.webofknowledge.com) and Cochrane library (www.cochranelibrary.com) to identify papers published between 1 January 1980 (Date of first publication of maternal vitamin D status) to 17 June 2018 following PRISMA guidelines.

Inclusion criteria were: articles reporting quantitative body composition measures or qualitative categories of BMI and vitamin D status (as 25(OH)D concentrations), data from any study design, published in the English language and including data on healthy pregnant women and their infants.

Searches were conducted using the following combinations of keywords or Medical Subject Heading (MeSH) phrases truncation where appropriate: Obes* OR BMI OR Body mass index AND Vitamin D OR 25-hydroxy vitamin D OR 25(OH)D OR Cholecalciferol OR calciferol OR calcifediol AND Pregnan* OR Maternal OR Mothers OR women. In a secondary search, bibliographies of selected articles were also examined for additional studies which may not have been identified by previous searches.

Selection criteria

After duplicate articles were removed and filter applied, two independent reviewers (BM, AMcG) screened the titles and abstracts to select only those studies that met the inclusion criteria. The full texts of all articles were obtained before reviewing for eligibility. Articles were further excluded at this stage if they reported data: 1) from blood samples taken after delivery; 2) from non-pregnant women only; 3) from non-healthy pregnant women; 4) without 25(OH)D concentrations; 5) where BMI and 25(OH)D measurements were not matched appropriately; 6) where BMI was not measured; 7) within a literature review; 8) not presented as required or unable to be calculated as (mean±SD); 9) from abstract only or from protocol or 10) from cohorts that were repeated in another publication.

Where possible, data were obtained from the control (healthy) pregnancy group of studies investigating unhealthy populations and included within the review. Manuscript authors were contacted if data were not presented in a useful format (for example, if data were presented only in figures or data presented as median and range).

Quality assessment

The quality of final articles was assessed using 2 quality assessment tools for observational studies and RCT (Newcastle-Ottawa Scale, Cochrane methodology) based on 5 areas (overall quality, cohort selection, methods, results and risk of bias), with each area containing questions relevant to the quality of the article. Scores were given for each area as +1 for addressing the issue appropriately, -1 for not addressing

the issue and 0 if it was unclear. Studies were defined as being of satisfactory quality if they scored ≥ 7 and were therefore deemed suitable for inclusion.

Data extraction

Data extracted from the articles were compiled into Microsoft Office Excel 2016 data files. Information extracted included study design, geographic location, sample size, biomarker of 25(OH)D status, 25(OH)D measurement methods used and BMI data, and then categorised based on (WHO BMI classification) for individual paper. Study populations were defined into trimesters (first, second and third) according to gestational age (first trimester (≤12 weeks), second trimester (13-26 weeks) and third trimester (27-40 weeks)). Quantitative data (mean ±SD) for vitamin D status as 25(OH)D concentrations were extracted as nmol/L, and conversion from ng/ml to nmol/L was carried out where appropriate (ng/ml * 2.5). Quantitative data for BMI were extracted as kg/m^2. Where available, dietary vitamin D intake was also recorded, albeit it was not possible to assess the impact of dietary data within the scope of this review. Vitamin D intake was extracted as µg/d and conversion from IU/d to µg/d was carried out where appropriate (1 IU= 40µg).

The accuracy of the extracted data was checked and approved by an independent third reviewer (LC) to minimize any imputation errors.

Statistical methods

Vitamin D status and BMI data are presented as mean ±SD and classified into 4 levels of sufficiency: deficiency (25(OH)D <25 nmol/L), insufficiency (25-50 nmol/L), sufficiency (50-75 nmol/L) and optimal (≥75 nmol/L) (SACN, 2016). In addition, BMI data were categorised into 3 groups: normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2) (WHO, 2004).

For ease interpretation, data have been presented in 2 ways within this review; 1) by trimester of pregnancy, 2) by study type.

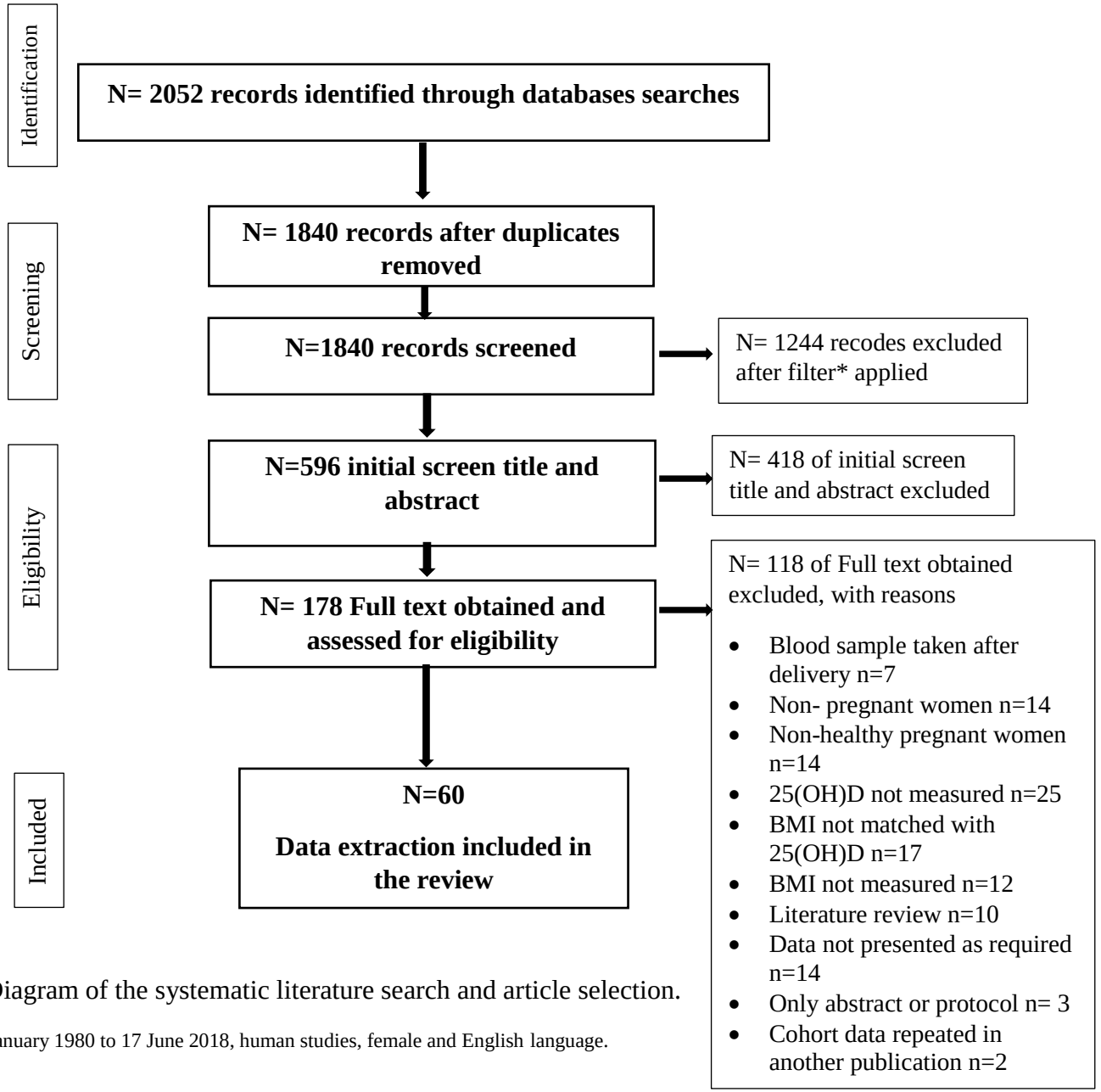

Figure 1. PRISMA Flow Diagram of the systematic literature search and article selection.

*****Filter criteria: Published from 01 January 1980 to 17 June 2018, human studies, female and English language.

Results

Identification of studies

A total of 2052 records were identified from the initial searches. After the removal of duplicates and filters were applied, a total of 596 records were screened by title and abstract with 418 of these papers excluded as they did not fit the inclusion criteria, leaving a total of 178 full-text articles which were obtained and assessed for eligibility. Of these 178 articles, 118 were excluded for reasons as outlined in Figure 1.

From this point forward the numbers within brackets e.g. (42, 36, 17) etc refer to the studies numbered within Tables 1-4. Data extracted for the purposes of this review were from 58 observational studies (Table 1, 2), and 2 randomised clinical trials (Table 3) and are presented in order of trimester of pregnancy.

The population of this review were pregnant women across all three trimesters. Within trimesters, findings are discussed in subsections of deficiency, insufficiency and sufficiency. A range of observational studies were included in this review and included prospective studies, retrospective analysis, cohort studies, case-control and cross-sectional studies. Measures of BMI were reported to be taken either pre-pregnancy or throughout pregnancy. The majority of the analyses for 25(OH)D used serum samples and a variety of different methods including 1.5% used immunodiagnostic system (IDS), 1.5% used enzyme immunoassay (EIA), 5% used high performance liquid chromatography (HPLC), 10% used chemiluminescence immunoassay (CLIA), 14% used radioimmunoassays (RIA), 20.5% used electrochemoluminiescent Immunoassay (ECLIA), 22 % used enzyme linked immunosorbent assays (ELISA) and the gold standard 25.5% used liquid chromatography tandem mass spectrometry (LC-MS/MS).

However, 2 studies (Hanieh *et al.*, 2015; Valkama *et al.*, 2018) did not report the methods used.

When studies were assessed according to trimester of pregnancy, a total of 14 reported vitamin D status in the first trimester, 5 studies covered a period between first and second trimesters, 21 studies were conducted during the second trimester, 4 covered a period between the second and third trimesters and 24 studies were conducted in the third trimester.

The studies reported in this review included 20 prospective studies, 1 retrospective analysis, 6 cohort studies, 12 case-control and 19 cross-sectional studies.

Discussion

The association between maternal BMI and vitamin D status during pregnancy according to trimester

First trimester

A total of 14 studies in this review reported vitamin D status in the first trimester of pregnancy (Table 1, a).

Deficiency

Only 1 study reported vitamin D deficiency (<25 nmol/L) (13.3 ±4.3nmol/L) at this time-point in a cohort of pregnant women from Saudi Arabia where the mean BMI was overweight albeit no significant associations were reported between BMI and vitamin D status. Although this study was conducted in a sunny country, the time of sampling was not reported and the cultural norm of long clothing coverage is common (4).

Insufficiency

The majority of studies (8, 23, 25, 33, 34, 39, 47) which assessed vitamin D status in the first trimester reported insufficient vitamin D status (25-50 nmol/L). These studies were from different regions, had a range of BMIs and were assessed across different seasons; seasonality was considered the most influential factor affecting vitamin D status. In a large cross-sectional study conducted in Malaysia, where the city climate is consistently hot and humid throughout the year, insufficient vitamin D status was observed among 396 normal weight pregnant women in first trimester. Increased

dietary vitamin D intake from food was found to reduce the prevalence of vitamin D insufficiency, and 55% of pregnant women were reported to meet the Malaysian Recommended Nutrient Intakes (RNI), of 5µg/d. In over half of those pregnant women who reportedly met the RNI, insufficient vitamin D status was observed (8). In addition, among 500 Korean pregnant women recruited prospectively during the year, the mean BMI was categorised as normal and the mean vitamin D status insufficient, which was related to a low daily vitamin D intake of 2.8µg/d (33). Furthermore, a study in London retrospectively analysed 346 blood samples collected from pregnant women at their initial booking visit and reported insufficient vitamin D status, with an independent association found between BMI and vitamin D, with obese pregnant women (BMI $\geq$ 30kg/m^2) having significantly lower vitamin D status than non-obese pregnant women. Seasonal variations were apparent with 30% of pregnant women having a lower vitamin D status between January and March compared to between July and September (23). A negative association was reported between BMI in the first trimester and maternal vitamin D status among 158 control pregnant women recruited in a case -control study (25). In another study based in London among 995 pregnant women, it was reported that maternal vitamin D status in the first trimester decreased with increasing BMI and vitamin D status increased in summer (34). Insufficient vitamin D status was reported among overweight pregnant women living in Amsterdam with low level of education, albeit 55% of sampling in this study was done during the winter months (39). Although insufficient vitamin D status was reported in 301 Pakistani pregnant women (47), information on vitamin D dietary intake and season of sample collection was not available. Furthermore, none of these studies reported information on vitamin use (25, 34, 39, 47).

Sufficiency

Four out of fourteen studies (27, 31, 40, 43) reported sufficient vitamin D status (>50 nmol/L) during the first trimester of pregnancy and included populations from Southampton (UK) (27), Boston (USA) (31), South-western Sweden (40) and Warsaw (Poland) (43). In the Southampton prospective study where the mean BMI was classified as overweight, 37% of pregnant women reported taking vitamin D supplements and supplementation use was correlated with vitamin D status. It was also reported that higher weight gain during pregnancy was associated with reduced 25(OH)D concentrations (27). In Warsaw, 80% of all pregnant women reported taking multivitamins containing vitamin D in the first trimester and overweight pregnant women had significantly lower vitamin D status than normal weight women in both winter and summer. In contrast to other studies, there was no significant difference in vitamin D status between winter and summer time, suggesting that high supplement use prevented seasonal variations in status (43). In a case-control study of pregnant women living in Boston Massachusetts, healthy pregnant women who were categorised as normal weight in the first trimester had sufficient vitamin D status and vitamin D status was inversely associated with BMI. However, there was no information reported about time of sampling (season) or vitamin use (31). In a multi-ethnic population-representative Swedish cohort of 1829 pregnant women in the first trimester where vitamin D status was sufficient, the mean BMI was classified as normal weight, 43% of pregnant women reported taking vitamin D supplements and 26% of pregnant women had travelled to regions with latitudes <35˚ in first trimester (40).

Between first and second trimesters

Few studies (2, 9, 48, 49, 50) assessed vitamin D status during pregnancy at a time, which fell between first and second trimesters.

Insufficiency

Insufficient vitamin D status was reported among women who were classified as being obese and obese class 2 pre-pregnancy compared to non-obese in Canada, with vitamin D status being inversely correlated with pre-pregnancy BMI (48). This was also observed in a cohort carried out across all seasons in North Carolina, when women classified as being overweight pre-pregnancy had insufficient vitamin D status (50).

Sufficiency

Vitamin D status was sufficient among all BMI groups in 2 studies albeit information on diet or vitamin D supplementation use was not reported in one study (2), whilst a study conducted in Pittsburgh, USA reported high regular use of multivitamins or prenatal vitamins during pregnancy (9). Almost optimal vitamin D status has been observed in normal weight and overweight pregnant women and sufficient vitamin D status in obese women, even where there was no variation in season of sampling however, no information was reported on supplement use (49).

Second trimester

A total of 21 studies assessed vitamin D status during the second trimester (Table 1, b).

Deficiency

Three of these studies reporting a high prevalence of vitamin D deficiency (13, 35, 36). In 30 Turkish pregnant women used as a control in a controlled cross-sectional study, deficient vitamin D status was reported and the mean BMI during second trimester was 26.1 kg/m^2, classified as overweight (13). In addition, a prospective study conducted in North India among 304 pregnant women, observed a high rate of vitamin D deficiency (92%), with high BMI during second trimester being a negative predictor of low vitamin D status in this cohort (36). These findings are similar to those in a Japanese pregnant cohort, which reported that higher pre-pregnancy BMI was negatively associated with vitamin D status (35). In contrast to the Turkish and Indian studies, the study on Japanese women reported a mean daily dietary vitamin D intake of 6.3µg/d, with 14% reportedly taking vitamin D supplements, whilst dietary intake and supplement use was not reported in the other studies.

Insufficiency

Insufficient vitamin D status in the second trimester was reported in 4 studies. Firstly, in a cross-sectional study of 1695 Chinese pregnant women where the mean BMI during second trimester was categorised as normal, vitamin D status was negatively correlated with BMI and body weight (37). This finding was not reported in multi-

ethnic population study in Norway, where the mean pre-pregnancy BMI was normal (52) or in a large prospective observational study of 370 Chinese pregnant women, where the mean pre-pregnancy BMI was normal (42). In a Korean pregnant cohort vitamin D status reportedly increased from first trimester to second trimester however, it was still considered insufficient and information was not reported on supplement use or the seasons of blood sampling. After the first visit women were advised to engage in outdoor physical activity and eat variety of foods specially fortified milk, which may explained the increase in vitamin D status (33).

Sufficiency

The majority of studies carried out during the second trimester reported sufficient vitamin D status (1, 6, 15, 18, 21, 28, 29, 36, 42, 43, 46) although these studies were conducted in sunny countries and in women who had a normal BMI. One Australian cohort of obese pregnant women where the mean vitamin D status was considered sufficient, it was noted that increased BMI during second trimester in winter and spring was a significant predictor of vitamin D deficiency (15). Furthermore, in a cross-sectional study of 201 pregnant women, whilst mean vitamin D status was considered sufficient and the mean BMI during second trimester classed as overweight, 59% reported no supplement use, with only 12 participants achieving a dietary intake of 10µg/d. The authors reported that ethnicity, season, BMI and supplement use were significant predictors of vitamin D status in the second trimester (29). In Chinese pregnant women where the mean BMI was normal, 73% of pregnant women reported regularly use vitamin D supplements, along with reports of seasonal variation in vitamin D status in pregnant women in second trimester (42).

In studies conducted in Europe (Warsaw, Amsterdam, Izmir, New Zealand) sufficient vitamin D status during the second trimester was reported. In the Karlsson *et al.*, (2013) study normal weight women were reported to have a sufficient vitamin D status with obese women almost reaching sufficiency (58.2 and 49.7 nmol/L, respectively); while dietary vitamin D intake (7.9 vs. 8.2 µg/d) and supplement use (44% vs. 47%) were not significantly different between the 2 groups (46). Furthermore, a study of 50 normal weight pregnant women living in Warsaw reported sufficient vitamin D status, albeit 70% of the cohort reported regular use of vitamin D supplements (43). Sufficient vitamin D status was also reported in a large prospective cohort conducted in New Zealand (1710 pregnant women) where the mean BMI in second trimester was classified as normal, blood sampling was carried out across all seasons and over half of women reporting consumption of vitamins contain vitamin D (51). Studies in second trimester that have reported the overall mean of vitamin D status as sufficient suggest that vitamin D deficiency is more likely if sampling occurred in winter, if women had a higher pre-pregnancy BMI or were not regularly users of vitamin D supplements (21, 6).

Sufficient vitamin D status in the second trimester was also reported in United States, in small sample size of 36 obese pregnant women living in Pittsburgh compared to optimal vitamin D status reported in non-obese pregnant women however, there was no association observed between obesity and vitamin D (38). Furthermore, in a cross-sectional study of 193 pregnant women where the mean BMI was categorised as overweight, a high vitamin D intake from supplements of 8.3µg/d was reported (28). Moreover, sufficient vitamin D status was also reported in 658 pregnant women living Seattle and Tacoma with a normal mean BMI (18). Vitamin D data on supplement use

and dietary intake were not reported in these studies (18, 38) and none of these studies reported on season of sampling (18, 28, 38).

Optimal levels of vitamin D (≥75 nmol/L) in the second trimester has been achieved in some studies around the world (7, 17, 38, 41, 42) with the mean BMI category of most of these cohorts being normal weight. This was the case in 498 pregnant women living in Seattle, 114 pregnant women living in Tacoma, 93 non-obese pregnant women living in Pittsburgh and 637 Chinese pregnant women. A high rate of vitamin D supplement use of 92% and 73 % was reported in Tacoma and in China respectively (41, 42) however, no information was reported on supplement use or season of sampling in other cohorts (17, 38). Vitamin D status in the second trimester was inversely associated with maternal adiposity as estimated by pre-pregnancy BMI, were the mean BMI of healthy control pregnant women was normal weight (41). Only 1 study of 537 overweight Canadian pregnant women in the second trimester showed a significant relationship between maternal vitamin D status and dietary intake from (diet, supplements) where the mean vitamin D intake diet and supplements was 20.3µg/d (7).

Between the second and third trimesters

Four studies (32, 53, 54, 55) assessed vitamin D status during pregnancy at a time, which fell between the second and third trimesters.

Insufficiency

Insufficient vitamin D status was reported in a large cohort of Chinese pregnant women where the mean BMI was categorised as normal weight, it has been reported that pregnant women who were overweight before pregnancy had significantly lower vitamin D status than non-overweight. Chinese pregnant women were non-supplements users of calcium and vitamin D as it was one of exclusion criteria however, there was a seasonal variation in maternal vitamin D status (55).

Sufficiency

Sufficient vitamin D status was reported among healthy control pregnant women in these cohorts where the mean BMI was categorised as normal weight, except Turkish women who were classified as obese. However, all Turkish pregnant women were non-supplements users of calcium and vitamin D as it was one of exclusion criteria in this study whereas, 30% of Hungarian pregnant women were taking vitamin D supplements and 44% of the samples were taken in spring and summer (32, 54). In addition, optimal vitamin D status was reported in Indian pregnant women and there was a statistically significant negative linear correlation between vitamin D status and BMI with a 26% rate of obesity in the control group (53).

Third trimester

Twenty-four studies assessing vitamin D status in the third trimester are included in this review (Table 1, c) and none of these studies reported vitamin D deficiency.

Insufficiency

Six studies reported insufficient vitamin D status in third trimester (11, 19, 24, 33, 56, 57), 3 studies reported the mean pre-pregnancy BMI being as normal weight (11, 24, 57) one included 95 pregnant women living in Sweden, another 60 pregnant women living Greece and finally 767 pregnant women living in Maastricht. It was reported that the main determinates of vitamin D status in the third trimester were season and use of vitamin D supplements, as the mean vitamin D intake from supplements was 5.8µg/d and from diet was 6.1µg/d (11) and the mean estimated vitamin D intake from food only was 10.5µg/d which is higher than many countries (24). However, none the pregnant women living in Greece were taking vitamin D supplements, and supplement usage was not reported in the Maastricht cohort; the author explained the insufficient vitamin D status could be related to reduced adherence to vitamin D supplementation or seasonal variations (57).

Insufficient vitamin D status was reported among pregnant women in the third trimester who were classified as overweight (19, 33, 56), including among 202 pregnant women living in Norway, 500 Korean pregnant women and among 70 Greek pregnant women. There was no significant association reported between early maternal BMI and vitamin D status in third trimester (19, 56). None of Greek pregnant women were taking a vitamin D supplement and there was no information on supplement use reported in either studies. Vitamin D status varied significantly with season in Norway however, season was not a factor in Greece as the study was conducted only in summer time.

Sufficiency

The majority of the studies (3, 5, 9, 10, 12, 16, 20, 27, 40, 43, 45, 46, 52) reported sufficient vitamin D status in third trimester of pregnancy around the world. Five studies reported the mean BMI of pregnant women in early pregnancy (5, 16, 43, 46, 17), 2 studies reported the mean before early pregnancy BMI (20, 52) and 1 study reported the mean BMI of pregnant women in third trimester (45) where the mean BMI of these cohorts were classified as being normal weight. Most of these studies reported vitamin D supplement use in 31%- 88% of pregnant women (5, 16, 20, 40, 43, 45, 46) but one study, conducted in multi-ethnic population in Norway of 719 pregnant women, did not report vitamin D supplementation. However, vitamin D status increased from insufficient status in second trimester to sufficient status in third trimester; this increase might be related to recommending vitamin D supplementation after the first visit; no information was reported on seasons of sampling (52). In a representative Swedish cohort of 1829 pregnant women, 17% of women travelled to regions with latitudes <35˚ in the third trimester. No significant association was observed in gestational weight gain and BMI with change in vitamin D status (40) however, other studies did not report on these associations.

Within The Southampton Women's Survey (SWS), a prospective cohort study, two studies assessed vitamin D status in third trimester and reported sufficient vitamin D status despite only 22% of women reporting supplement use and the mean pre-pregnancy BMI being classified as overweight (10, 27).

Two studies reported sufficient vitamin D status in the third trimester and the mean pre-pregnancy BMI or BMI in third trimester was considered as obese (3, 12). Among 206 pregnant women was living in Oakland, where the mean vitamin D intake from

prenatal vitamins was 19µg/d, 71% of pregnant women reported daily supplement use. It has been reported that maternal vitamin D was significantly associated with pre or early pregnancy BMI (12). This was also observed among 40 healthy control pregnant women however, no information on supplement use or the season of sampling was available (3).

Sufficient vitamin D status in the third trimester was reported for 384 pregnant women from different BMI groups from a prospective cohort conducted in Pittsburgh, Magee USA, where the majority of pregnant women used multivitamins or prenatal vitamins regularly during the last 3 months of pregnancy (9).

Optimal vitamin D status in third trimester was reported in 5 studies from different geographical locations (14, 22, 26, 44, 58). In a cross sectional study of 38 normal weight and 23 obese pregnant women living in Chicago, both groups reported prenatal vitamin use and no significant difference was reported in vitamin D status between normal weight and obese pregnant women (26). Two studies reported optimal vitamin D status where the mean pre-pregnancy BMI was overweight in 37 and 40 healthy pregnant women who were used as controls in case control studies which were conducted in Ontario, Canada and in South Carolina respectively (22, 44). In the Canadian cohort the mean vitamin D from dietary intake (food and supplement) was 14.4µg/d, while this study was conducted in wintertime to minimize the sun light influence (22), no information reported on season of sampling and vitamin D supplement use in South Carolina study (44). A study comprising 910 pregnant women living in Singapore where the mean BMI in the third trimester was overweight, reported optimal vitamin D status with a high rate of participants taking vitamin D supplementation 74% during pregnancy, the climate of the region is sun-rich all year round (58).

Optimal vitamin D status in third trimester was reported among 135 Egyptian pregnant women living Cairo, where the mean maternal BMI in the third trimester was classified as obese. Pregnant women were not taking any form of calcium or vitamin D supplements during the last 3 months of pregnancy in this study. Maternal vitamin D status had significant negative correlations with BMI and maternal age, and significant positive correlations with fish consumption and skin exposure (14).

The association between maternal BMI and vitamin D status during pregnancy according to study type

Prospective studies

A total of 20 prospective studies reported vitamin D status in different trimesters and BMI categories (Table 2, a). Vitamin D deficiency (<25 nmol/L) was only observed in 1 prospective study conducted in North India among pregnant women, in which 92% of pregnant women were classified as vitamin D deficient and BMI during second trimester was a negative predictor of vitamin D status (36).

Insufficient vitamin D status (25-50 nmol/L) was reported in 7 prospective studies, during the first trimester where the mean BMI was classified as normal weight and overweight in two populations (London and Pakistan) (34, 47). These studies reported that maternal vitamin D status in the first trimester decreased with increasing BMI and vitamin D status increased in summer (34); none of these studies reported information on vitamin use (34, 47). During the second and third trimesters, insufficient vitamin D status was reported among pregnant women who had pre-pregnancy BMI categorised as normal weight (42, 57). The authors suggested that the insufficient vitamin D status could be related to reduced adherence to vitamin D supplementation or owing to seasonal variation (57). Insufficient vitamin D status was also reported among Norwegian pregnant women in the third trimester who were classified as overweight, and the authors reported seasonal fluctuations in vitamin D status (19). Whilst these prospective studies assessed vitamin D status and measured BMI, the researchers did not investigate potential associations between vitamin D status and BMI (42, 57) and in 1 study where these associations were examined, no significant associations were

found (19). Furthermore, insufficient vitamin D status has been observed across all trimesters of pregnancy in obese pregnant women compared to normal weight who had sufficient vitamin D status despite obese women reporting higher dietary intakes. Percentage fat mass in addition to BMI has also been shown to be negatively associated with vitamin D status in all trimesters of pregnancy (46). In a cohort of Korean pregnant women recruited prospectively throughout the year, the mean BMI was categorised as normal and vitamin D status reportedly increased as pregnancy progressed albeit concentrations were still considered insufficient at all time-points; season of sampling was not taken into consideration. The rise in vitamin D status may be owing to advice given on increasing outdoor physical activity and consumption of fortified milk (33).

Sufficient vitamin D status (>50 nmol/L) has been reported in most prospective studies included in this review (9, 10, 16, 18, 21, 27, 38, 46, 49, 51, 52). Four studies had assessed vitamin D status at more than one time-point during pregnancy and reported sufficient vitamin D status (27, 9, 52, 46, 49). During the first and third trimesters in the Southampton prospective study where the mean BMI was classified as overweight, 37% of pregnant women reported taking vitamin D supplements and supplementation use was correlated with vitamin D status. In this study it was also reported that higher weight gain during pregnancy was associated with reduced 25(OH)D concentrations (27). These findings are comparable to studies conducted in Pittsburgh and Finland where sufficient vitamin D status was observed among all BMI groups in all trimesters but obese women had significantly lower vitamin D status compared to non-obese women (9, 49). In both studies, vitamin D status did not change as pregnancy progressed (27, 9). In addition, in a multi-ethnic population study in Norway, where the mean pre-pregnancy BMI was normal, vitamin D status increased from insufficient

status in second trimester to sufficient status in third trimester; albeit this increase is most likely due to increased use of vitamin D supplementation following recommendations at the first visit. No information was reported on associations between maternal BMI and vitamin D status or on seasons of sampling (52).

Sufficient vitamin D status was also reported in 7 prospective studies which assessed vitamin D status at one time point during pregnancy in women classified as normal weight (18, 21, 51) and 1 study reported significant negative associations between pre-pregnancy BMI and vitamin D status (21).

A small study conducted in Pittsburgh, USA reported significantly lower vitamin D status in obese compared to non-obese pregnant women but found no significant association between BMI and vitamin D status (38). Similar null associations between BMI and vitamin D status in third trimester have been reported in a Southampton population but were not investigated in Vietnam populations (10, 16).

Optimal vitamin D status (≥75 nmol/L) was observed during the second and third trimesters in 3 prospective studies (7, 38, 58) of pregnant women who had a mean BMI classified as overweight (7, 58). The high vitamin D status observed was most likely owing to high dietary intakes (as high as 20.3µg/d in Canada) (7) or high vitamin D supplement use (58). It has been reported that pregnant women who had vitamin D status (≥75 nmol/L) had lower BMI (58).

Three prospective studies assessed vitamin D status in cord blood (9, 19, 47) and reported insufficient vitamin D status in both the mother and in cord blood samples (19, 47), with maternal status significantly predicting cord blood status (19). In 1 study, vitamin D status in cord blood was significantly higher in neonates born to non-obese mothers compared to those born to obese mothers, possibly owing to lower

maternal vitamin D status in obese mothers (9). Furthermore, in this study, it was observed that an increase in BMI from 22 to 34 weeks gestation, resulted in a 2.1-fold increase in neonatal vitamin D deficiency (9).

Retrospective analysis

A study in London retrospectively analysed 346 blood samples collected from pregnant women at their initial booking visit and reported insufficient vitamin D status (Table 2, b), with an independent association found between BMI and vitamin D status. Obese pregnant women (BMI $\geq$ 30kg/m^2) had significantly lower vitamin D status than non-obese pregnant women and seasonal variations were apparent with 30% of pregnant women having a lower vitamin D status between January and March compared to between July and September, irrespective of body weight (23).

Cohort studies

A total of 6 cohort studies reported vitamin D status of pregnant women within different trimesters and across BMI categories and are included in this review (Table 2, c). None of these studies reported vitamin D deficiency (<25 nmol/L) whilst, insufficient vitamin D status (25-50 nmol/L) was reported in 2 of the cohort studies of pregnant women with mean pre-pregnancy BMI classified as overweight (39, 50). However, there was no association reported between maternal BMI and vitamin D status (39, 50). Furthermore, insufficient vitamin D status was reported in a large cohort of Chinese pregnant women and it was found that those women who were overweight before pregnancy had significantly lower vitamin D status than normal weight women (55).

Sufficient vitamin D status (>50 nmol/L) has been reported in 4 cohort studies within different BMI categories (15, 39, 40, 43), and one study reported that increasing BMI was a significant negative predictor of vitamin D status (15). Furthermore, in a multi-ethnic population-representative Swedish cohort of 1829 pregnant women with mean BMI classified as normal weight, vitamin D status increased from first to third trimester albeit status at both trimesters was within range of sufficiency. This increase was related to factors such as dietary vitamin D intake, vitamin D supplementation and travel to regions with latitudes <35˚. There were no significant associations between gestational weight gain or BMI and change in vitamin D status (40). In Warsaw, a cohort study of pregnant women with normal BMI reported sufficient vitamin D status across all trimesters of pregnancy, with overweight pregnant women having significantly lower vitamin D status than normal weight women in both winter and summer. In contrast to other studies, there was no significant difference in vitamin D status between winter and summer time, suggesting that high supplement use prevented seasonal variations in status (43).

Case-control studies

There are 12 case-control studies included in this review, where vitamin D status of the healthy control group has been reported from each study (Table 2, d). Vitamin D deficiency (<25 nmol/L) was observed only in one study among Turkish pregnant women in the second trimester where the mean BMI was classified as overweight, however, there was no association reported between maternal BMI and vitamin D status (13). Insufficient vitamin D status (25-50 nmol/L) was observed in 2 healthy control pregnant women groups, firstly during the first trimester where the mean BMI

of women was classified as being overweight and secondly at the time between the first and second trimesters where the mean pre- pregnancy BMI was classified as being obese and obese class 2. Both of these studies reported a significant negative association between maternal BMI and maternal vitamin D status (25, 48).

The majority of case-control studies included in this review reported sufficient vitamin D status (>50 nmol/L) in healthy control groups of pregnant women within different trimesters across BMI categories (3, 5, 31, 32, 45, 48, 54), with some studies reporting significant negative associations between BMI and vitamin D status (31, 48) whilst others did not find these associations (32, 54). Other studies did not investigate links between maternal BMI and vitamin D status despite having measured both parameters (3, 5, 45).

Optimal vitamin D status (≥75 nmol/L) was observed among healthy control pregnant women in some studies around the world (22, 41, 44) and reported significant inverse associations between vitamin D status and BMI in normal weight pregnant women (41). In comparison, studies where the mean pre-pregnancy BMI was overweight (22, 44), and higher dietary intakes (from food and supplements) were reported, no significant correlations was found between maternal BMI and vitamin D status (22) or were not investigated (44).

Only 1 case-control study reported vitamin D status of cord blood samples, and reported that neonates born to healthy control mothers had sufficient vitamin D status where the mothers had optimal vitamin D status at third trimesters (22).

Cross-sectional studies

A total of 19 cross-sectional studies included in this review reported vitamin D status within different trimesters and across BMI categories (Table 2, e). Two studies reported deficient vitamin D status (<25 nmol/L) during the first and second trimester among Saudi pregnant women where the mean BMI was overweight and among Japanese pregnant women with mean pre-pregnancy BMI considered normal. However, pre-pregnancy BMI was positively correlated with vitamin D status in the Japanese study but not significantly associated in Saudi study (4, 35).

Insufficient vitamin D status (25-50 nmol/L) was observed in 6 cross-sectional studies of pregnant women (8, 11, 24, 29, 37, 56). A cross-sectional study conducted in Malaysia, where the city climate is consistently hot and humid throughout the year, reported insufficient vitamin D status among normal weight pregnant women in first trimester. Increased dietary vitamin D intake from food was found to reduce prevalence of vitamin D insufficiency. However, pre-pregnancy BMI in this study was not significantly associated with hypovitaminosis D (8). Two studies conducted during the second trimester of pregnancy also reported insufficient vitamin D status despite being from two different populations (China and Australia) and different BMI groupings (normal weight and obese/obese class 2 pregnant women). Both studies reported significant negative correlations between BMI and vitamin D status (29, 37). Furthermore, insufficient vitamin D status has also been observed during second and third trimesters in pregnant women of normal weight and overweight BMI, although no significant associations were found between BMI and vitamin D status (11, 56) or was not investigated (24).

The majority of cross-sectional studies included in this review reported sufficient vitamin D status (>50 nmol/L) within all trimesters across different BMI categories (1, 2, 6, 12, 20, 28, 29, 30). Three studies included a range of BMI categories (normal

weight, overweight, obese) and reported sufficient vitamin D status across pregnancy and found that vitamin D status was inversely correlated with early pregnancy or pre-pregnancy BMI (2, 29, 30). Similar associations between BMI and vitamin D status were also reported in 2 studies which recruited pregnant women with a mean BMI classified as overweight and obese (28, 12). In contrast, 3 studies where the mean BMI was classified as normal, did not investigate the associations between maternal BMI and vitamin D status (1, 6, 20).

Optimal vitamin D status (≥75 nmol/L) was observed in the second and third trimesters across BMI categories (14, 17, 26, 53). One study of Indian pregnant women where the mean BMI was classified as normal, reported a statistically significant negative linear correlation between vitamin D status and BMI with a 26% rate of obesity in the control group (53). However, not all studies have found significant associations between BMI and vitamin D status (17, 26).

Vitamin D status of cord blood has been measured in 5 cross sectional studies (12, 14, 24, 26, 56). Insufficient vitamin D status was reported in cord blood samples of neonates born to normal weight or overweight mothers who had insufficient vitamin D status (24, 56) and also among cord samples of neonates born to obese mothers who had sufficient or optimal vitamin D status (12, 14). It has been observed that neonates of obese mothers have significantly lower cord vitamin D status compared to neonates of normal weight mothers despite similar maternal vitamin D status concentrations at the end of pregnancy (26). Overall, maternal and cord vitamin D status are determined by season of delivery, vitamin D dietary intake, skin pigmentation, supplement use and BMI (12, 14, 26).

Vitamin D supplementation during pregnancy

Only 2 RCT studies met the inclusion criteria for this review (1, 2) (Table 3). The first one from Kashan, Iran, had two groups of pregnant women who were recruited in the second trimester (n=24 in each group); the treatment group received 10µg/d vitamin D_3 supplement and the control group received a placebo supplement for 9 weeks. The mean BMI of both groups was classified as overweight and there were no significant differences in dietary intake between the two groups. Following supplementation, vitamin D status was significantly higher in the treatment group compared to the placebo group and supplementation was reported to have beneficial effects on metabolic status including a decrease in high-sensitivity C- reactive protein and insulin concentrations. BMI was not considered in any analysis for this RCT (1). In the second RCT from Cork, Ireland, 3 groups of pregnant women (n= 48 in each group) at 14 gestational weeks were recruited, with the treatment groups receiving supplements containing 10µg/d vitamin D_3 or 20µg/d vitamin D_3 and with the control group receiving a placebo from 14 weeks gestation until delivery. The mean BMI of the 10µg/d vitamin D_3 group and placebo group was classified as overweight while 20µg/d vitamin D_3 group was classified as normal weight and there were no significant differences in dietary intake or vitamin D status at baseline between the three groups. After supplementation, vitamin D status at 24 and 36 weeks gestation increased in all groups with a significantly higher increase in the treatment groups than placebo, the increase in vitamin D status observed in the placebo group was attributed to seasonal variability and the larger increase in the 20µg group may well have been due to the normal BMI, where normal weight participants are known to have a higher response to vitamin D supplementation then their overweight or obese counterparts. No

association was observed between maternal BMI at baseline and maternal vitamin D at baseline or change of vitamin D status after intervention (2).

Vitamin D status in cord blood

Some observational studies (9, 12, 14, 19, 22, 24, 26, 47, 56) did assess vitamin D status in cord blood samples (Table 1, d) and reported that neonates of obese mothers have significantly lower cord vitamin D status compared to neonates of normal weight mothers (26). It has been observed that an increase in BMI from 22 to 34 weeks gestation, results in a 2.1-fold increase in neonatal vitamin D deficiency (9). Overall, maternal and cord vitamin D status are determined by season of delivery, vitamin D dietary intake, skin pigmentation, supplement use and BMI (12, 14, 26). In a Norwegian study in overweight women in early pregnancy, no significant association between maternal BMI and cord blood vitamin D status was reported despite there being a strong positive association between maternal vitamin D status and cord blood vitamin D status (19). Three studies, 2 in Greece and 1 in Pakistan reported that both the mother and neonate had insufficient vitamin D status; vitamin D status in mothers was not significantly higher than the neonates (24, 47, 56). In addition, it has been observed that neonates born to a mother who had deficient vitamin D status during pregnancy had significantly lower vitamin D status compared with neonates born to mothers who had sufficient vitamin D status during pregnancy (24).

Overall summary of studies divided the study cohort based on BMI categories

A total of 13 studies were included in this review which divided the study cohort based on BMI categories even if the association between maternal BMI and vitamin D was

not the main aim of the research; 6 studies reported data from the first trimester, 3 studies from second trimester, 4 studies which considered between first and second trimesters and 3 studies at third trimester. Only 2 study assessed vitamin D status in cord blood samples according to maternal BMI categorised by studies (Table 4). In this review, we have assessed the association between maternal BMI and vitamin D status during pregnancy within each trimester and classified status as deficiency, insufficiency and sufficiency. Obese or obese class 2 pregnant women in these studies had insufficient vitamin D status compared to non-obese (2, 9, 23, 29, 30, 39, 43, 46, 48). Only 2 studies reported optimal vitamin D status in normal weight and overweight pregnant women and sufficient vitamin D status in obese women, 1 in Pittsburgh with small participant numbers and where the BMI was measured pre-pregnancy and prenatal vitamin use was common in this population, however, no information reported on season of sampling (38). The other study was conducted in Finland, no significant difference was observed for the season of blood collection and no information was reported on supplement use (49). In a cross-sectional study among 1345 pregnant women living in Denmark, it was reported that a 5 unit increase in BMI was associated with a 25(OH)D lowering of 4.2 nmol/L and 2.8 nmol/L in winter and summer months, respectively (2). Across the first trimester, 25(OH)D concentrations during pregnancy were inversely correlated with maternal BMI (30, 43). Maternal BMI in the second trimester was a significant predictor of vitamin D status in a study of 201 Caucasian pregnant women living in Australia (29).

Furthermore, in a prospective pregnancy cohort study conducted in 398 pregnant white and black women, neonates of obese mothers had significantly lower 25(OH)D concentrations compared to neonates of lean mothers (49.9 vs. 56.2 nmol/L; $P < 0.05$) after adjustment for ethnicity, season, gestational age, multivitamin use, physical

activity, and maternal age. The authors concluded that obese women and their neonates are at a high risk of vitamin D deficiency despite maternal prenatal vitamin use (9). Moreover, a sufficient vitamin D status in the first trimester was reported in a prospective pregnancy cohort of Pakistani pregnant women where the cohort was divided into 3 groups in the first trimester BMI (underweight, normal weight and overweight), no information was reported on time of sampling (season) or vitamin use (47).

Strengths and limitations of this review

To the authors' knowledge this is the first systematic review to explore current knowledge on the association between maternal obesity and vitamin D status of the mother and their infants. Whilst there are some systematic reviews investigating associations between vitamin D status and effect of supplementation during pregnancy on maternal and infant health outcomes, the association between obesity and vitamin D status was not considered in these analyses (Thorne-Lyman and Fawzi, 2012;Harvey *et al.,* 2014;Faustino *et al.,* 2015). The majority of the studies included in this review were observational studies, highlighting the need for RCTs to assess the association between maternal obesity and maternal and child vitamin D status. Owing to the heterogeneity of the studies included in this review, a meta-analysis of the data was not possible and assessment of the magnitude of effect of BMI on vitamin D status could not be elucidated. However, we have adhered as much as possible to guidelines for reporting of systematic reviews.

Conclusion

Evidence shows that obese or obese class 2 pregnant women have consistently lower vitamin D status compared to non-obese pregnant women in studies across the globe and maternal BMI is negatively associated with maternal vitamin D status. As infant vitamin D stores are directly related to maternal stores, studies report that vitamin D status is lower in infants born to obese mothers compared to infants born to normal weight mothers. As maternal obesity was negatively associated with maternal and infant vitamin D status, with potential implications for both maternal and child health, public health advice on vitamin D supplementation during pregnancy should consider the implications of pre-pregnancy BMI.

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Table 1, a: Observational studies of vitamin D status in the first trimester

N	First author, year	Study design	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
4	Al-Ajlan, 2015	CS	515	Riyadh, Saudi Arabia 24.7136° N, 46.6753° E	N/A	11.2 ± 2.7wk	**1st trimester**	23.4 ± 15.7	ECLIA	28.4 ± 6.2	**Overweight**	
8	Bukhary, 2016	CS	396	West Peninsular Malaysia, Selangor 3.0738° N, 101.5183° E	1st January until end of April 2014	First trimester	**1st trimester**	27.7 ± 4.9	ECLIA	22.9 ±1.8**	**Normal weight**	
23	McAree, 2013	Retrospective analysis	227	North West London 51.5074° N, 0.1278° W	April 2008 and March 2009	At the initial booking visit	**1st trimester**	39.0 ± 12.7	LC-MS	<30 kg/m^2	**Normal weight & overweight**	
23	McAree, 2013	Retrospective analysis	43	North West London 51.5074° N, 0.1278° W	April 2008 to March 2009	At the initial booking visit	**1st trimester**	29.0 ± 10.9	LC-MS	≥30 kg/m^2	**Obese**	
25	Makgoba, 2011	CCS	158	Glasgow, UK 55.8642° N, 4.2518° W	N/A	~12.5 wk	**1st trimester**	47.6 ± 26.7	LC-MS/MS	25.2 ± 4.0	**Overweight**	
27	Moon, 2015	Prospective, cohort study	1753	Southampton, UK 50.9097° N, 1.4044° W	April 1998 to October 2002	11 wk	**1st trimester**	62.7 ± 25.9	LC-MS/MS	25.2 ±4.6**	**Overweight**	

30	Perez-Lopez, 2011	CS	502	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	67.8 ± 8.5	ECLIA	N/A	**N/A**	
30	Perez-Lopez, 2011	CS	307	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	70.2 ± 7.9	ECLIA	BMI <25 kg/m^2	**Normal weight**	
30	Perez-Lopez, 2011	CS	132	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	64.1 ± 9.0	ECLIA	BMI 25-30 kg/m^2	**Overweight**	
30	Perez-Lopez, 2011	CS	63	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	60.6 ±10.0	ECLIA	BMI ≥ 30 kg/m^2	**Obese**	
31	Powe, 2010	CCS	131	Boston, USA 42.3601° N, 71.0589° W	N/A	11.6 ± 3.0 wk	**1st trimester**	72.0 ± 2.0	LC-MS/MS	24.4 ± 4.5	**Normal weight**	
33	Park, 2014	Prospective study	500	Seoul, Korea 37.5665° N, 126.9780° E	May 2011 to July 2012	12-14 wk	**1st trimester**	32.0 ± 14.5	ECLIA	21.2 ± 2.8	**Normal weight**	
34	Savvidou, 2012	Prospective study	796	London, UK 51.5074° N, 0.1278° W	1 March 2006	11–13 wk	**1st trimester**	47.6 ± 11.7	LC-MS/MS	24.0 ± 1.4	**Normal weight**	Women had vaginal delivery
34	Savvidou, 2012	Prospective study	111	London, UK 51.5074° N, 0.1278° W	1 March 2006	11–13 wk	**1st trimester**	45.0 ± 14.2	LC-MS/MS	25.9 ± 1.8	**Overweight**	Women had emergency C- section
34	Savvidou, 2012	Prospective study	88	London, UK 51.5074° N, 0.1278° W	1 March 2006	11–13 wk	**1st trimester**	55.9 ± 14.6	LC-MS/MS	26.1 ± 1.6	**Overweight**	Women had Elective C- section

39	Gerrit van den Berg, 2013	Cohort study	372	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	49.8 ± 23.7	ELISA	BMI >25 kg/m^2 **	**Overweight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	58.3± 24.1	ELISA	BMI >25 kg/m^2 **	**Overweight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	62.6 ± 27.5	ELISA	BMI >25 kg/m^2 **	**Overweight**
39	Gerrit van den Berg, 2013	Cohort study	1902	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	63.6 ± 39.8	ELISA	BMI <25 kg/m^2 **	**Normal weight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	71.5± 28.4	ELISA	BMI <25 kg/m^2 **	**Normal weight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	69.1± 27.0	ELISA	BMI <25 kg/m^2 **	**Normal weight**
40	Bärebring, 2016	Cohort study	1829	South-western Sweden 57-58°N	2013 (2 September– 8 November) and 2014 (24 February– 13 June).	10.8 ± 2.0 wk	**1st trimester**	64.5 ± 24.5	LC-MS/MS	24.5 ± 4.2	**Normal weight**
43	Bartoszewicz, 2013	Cohort study	50	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 – – March 31) and summer (April 1 – September 30)	1st trimester	**1st trimester**	57.7 ± 22.2	ECLIA	23.4 ±3.1	**Normal weight**

46	Karlsson, 2014	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	11.9 ± 1.0 wk	**1st trimester**	64.2 ± 18.3	CLIA	22.0 ± 1.4	**Normal weight**	
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	11.5 ± 1.5 wk	**1st trimester**	49.7 ± 11.5	CLIA	33.9 ± 3.3	**Obese**	
47	Riaz, 2018	Prospective study	301	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.78 ± 2.30 wk	**1st trimester**	40.9 ± 32.3	ELISA	22.4 ± 4.6	**Normal weight**	All women
47	Riaz, 2018	Prospective study	63	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.54 ± 2.40 wk	**1st trimester**	43.7 ± 30.7	ELISA	17.2 ± 1.0	**Normal weight**	
47	Riaz, 2018	Prospective study	130	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.79 ± 2.25 wk	**1st trimester**	38.3 ± 29.4	ELISA	20.9 ± 1.3	**Normal weight**	
47	Riaz, 2018	Prospective study	108	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.92 ± 2.30 wk	**1st trimester**	42.4 ± 36.3	ELISA	27.3 ± 4.0	**Overweight**	
Observational studies of vitamin D status in the first & second trimesters												
2	Andersen, 2013	CS	36	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	59.7	LC-MS/MS	<18.5 kg/m^2	**Under weight**	
2	Andersen, 2013	CS	740	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	66.6	LC-MS/MS	18.5–24.9 kg/m^2	**Normal weight**	
2	Andersen, 2013	CS	300	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	62.6	LC-MS/MS	25–29.9 kg/m^2	**Overweight**	

2	Andersen, 2013	CS	81	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	56.7	LC-MS/MS	30–34.9 kg/m^2	**Obese**
2	Andersen, 2013	CS	37	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	52.1	LC-MS/MS	>35 kg/m^2	**Obese class 2**
9	Bodnar, 2007	Prospective cohort	223	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	62.8(55.0, 70.4)*	ELISA	<25 kg/m^2**	**Normal weight**
9	Bodnar, 2007	Prospective cohort	87	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	58.6 (51.5, 66.8)*	ELISA	25–29.9 kg/m^2**	**Overweight**
9	Bodnar, 2007	Prospective cohort	82	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	55.9 (48.7, 64.2) *	ELISA	≥30 kg/m^2 **	**Obese**
48	Woolcott, 2016	CCS	1002	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	54.6 ± 16.8	CLIA	<25 kg/m^2**	**Normal weight**
48	Woolcott, 2016	CCS	311	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	51.0 ± 15.6	CLIA	25-<30 kg/m^2**	**Overweight**
48	Woolcott, 2016	CCS	124	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N	2002–2010	12-15 wk	**1st & 2nd trimester**	48.2 ± 16.5	CLIA	30-<35 kg/m^2**	**Obese**

				63° 34' W Canada							
48	Woolcott, 2016	CCS	84	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	45.7 ± 15.4	CLIA	≥35 kg/m^2**	**Obese class 2**
49	Valkama, 2018	Prospective study	43	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	74 ± 23	N/A	<25 kg/m^2	**Normal weight**
49	Valkama, 2018	Prospective study	34	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	73 ± 32	N/A	25-<30 kg/m^2	**Overweight**
49	Valkama, 2018	Prospective study	84	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	64 ± 24	N/A	30-<35 kg/m^2	**Obese**
49	Valkama, 2018	Prospective study	58	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	57 ± 22	N/A	≥35 kg/m^2	**Obese class 2**
50	Benjamin Neelon, 2018	Cohort study	476	Durham, North Carolina 35.9940° N, 78.8986° W	July 2009 to December 2011	13.2 ± 5.5 wk	**1st & 2nd trimester**	41.1 ± 14.2	IDS	27.4 ±7.0**	**Overweight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
CS- cross section, CCS-case control study, N/A not available, wk- week gestational.
* n 9 study vitamin D presented as mean (95% CI)
** Pre-pregnancy BMI

Table 1, b: Observational studies of vitamin D status in the second trimester

N	First author, year	Study design	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
1	Allen, 2013	CS	98	Western Australia 27.6728° S, 121.6283° E	1989 to 1991	18 wk	**2nd trimester**	55.0 ± 17.1	ELISA	22.6 ± 4.4	**Normal weight**	
1	Allen, 2013	CS	428	Western Australia 27.6728° S, 121.6283° E	1989 to 1991	18 wk	**2nd trimester**	58.6 ± 16.3	ELISA	21.8 ± 3.7	**Normal weight**	
6	Mehmet Bal, 2016	CS	50	İzmir, Turkey 38.4237° N, 27.1428° E	All blood sampling summer season	24-28 wk	**2nd trimester**	63.5 ± 23.2	LC-MS/MS	22.8 ± 1.6	**Normal weight**	
7	Aghajafari, 2016	Prospective cohort	537	Alberta, Canada 53.9333° N, 116.5765° W	March 2009 to July 2012	Second trimester	**2nd trimester**	95.3 ± 25.0	LC-MS/MS	26.0 ± 5.0	**Overweight**	
13	Daglar, 2016	CCS	30	Ankara, Turkey 39.9334° N, 32.8597° E	March 2014 to May 2014	19.4 ± 4.6 wk	**2nd trimester**	22.7 ± 18.2	ELISA	26.1 ± 5.2	**Overweight**	
15	Davies-Tuck, 2015	Cohort study	1550	Monash, Australia 37.9016° S, 145.1155° E	July 2009 to June 2010	13.7± 3.3 wk	**2nd trimester**	71.0 ± 47.9	ECLIA	31.2 ± 13.5	**Obese**	
17	Huang, 2014	CS	498	Seattle, Washington. 47.6062° N, 122.3321° W	April 2009 to December 2010	At or prior to 20 wk	**2nd trimester**	86.0 ± 21.7	LC-MS/MS	23.5 ±4.7**	**Normal weight**	

18	Huang, 2013	Prospective cohort	658	Washington. Seattle, Tacoma 47.6062° N, 122.3321° W	1996 to 2008	At or prior to 20 wk	**2nd trimester**	72.7 ± 21.0	LC-MS/MS	23.8 ±5.4**	**Normal weight**
21	Hrudey, 2016	Prospective cohort	1882	Amsterdam 52.3702° N, 4.8952° E	January 2003 to March 2004,	16.1 wk	**2nd trimester**	60.4 ± 0.7	ELISA	22.9 ± 3.7	**Normal weight**
28	Ozias, 2014	CS	193	Kansas, US 39.0119° N, 98.4842° W	January 2006 to November 2009	14 ± 4 wk	**2nd trimester**	56.7 ± 33.2	EIA	27.0 ±5.0**	**Overweight**
29	Perampalam, 2011	CS	100	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009.	24-28 wk	**2nd trimester**	61.3 ± 23.4	ECLIA	27.3 ± 5.7	**Overweight**
29	Perampalam, 2011	CS	33	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009.	24-28 wk	**2nd trimester**	65.9 ± 28.8	ECLIA	16.0 - 24.9 kg/m^2	**Normal weight**
29	Perampalam, 2011	CS	44	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009.	24-28 wk	**2nd trimester**	61.6 ± 21.2	ECLIA	25.0- 29.9 kg/m^2	**Overweight**
29	Perampalam, 2011	CS	15	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009.	24-28 wk	**2nd trimester**	53.4 ± 17.2	ECLIA	30.0- 34.9 kg/m^2	**Obese**
29	Perampalam, 2011	CS	8	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009.	24-28 wk	**2nd trimester**	60.7 ± 21.9	ECLIA	≥35.0 kg/m^2	**Obese class 2**

29	Perampalam, 2011	CS	101	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	69.5 ± 26.9	RIA	28.3 ± 5.8	**Overweight**	
29	Perampalam, 2011	CS	36	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	62.8 ± 24.3	RIA	16.0- 24.9 kg/m^2	**Normal weight**	
29	Perampalam, 2011	CS	29	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	58.9 ± 30.1	RIA	25.0- 29.9 kg/m^2	**Overweight**	
29	Perampalam, 2011	CS	21	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49.0 ± 17.4	RIA	30.0- 34.9 kg/m^2	**Obese**	
29	Perampalam, 2011	CS	13	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49.1 ± 25.0	RIA	≥35.0 kg/m^2	**Obese class 2**	
33	Park, 2014	Prospective study	500	Seoul, Korea 37.5665° N, 126.9780° E	May 2011 to July 2012	20-22 wk	**2nd trimester**	46.5 ± 23.6	ECLIA	22.6 ± 2.8	**Normal weight**	
35	Shiraishi, 2014	CS	284	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	17.0 ± 11.7	ECLIA	20.4 ±2.4**	**Normal weight**	All women
35	Shiraishi, 2014	CS	157	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	25.7 ± 12.7	ECLIA	20.3 ±2.3**	**Normal weight**	During summer
35	Shiraishi, 2014	CS	127	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	23.0 ± 10.5	ECLIA	20.6 ±2.6**	**Normal weight**	During winter

36	Singla, 2015	Prospective study	304	North India 20.5937° N, 78.9629° E	2010 to 2013	12–16 wk	**2nd trimester**	20.9 ± 4.5	ELISA	21.8 ± 3.4	**Normal weight**
37	Tao, 2012	CS	1695	Shanghai, China 31.2304° N, 121.4737° E	1 July 2008 to 30 June 2009	21.0 ± 6.2 wk	**2nd trimester**	43.9 ± 28.6	ECLIA	22.5 ± 3.1	**Normal weight**
38	Laura, 2013	Prospective cohort study	64	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	88.5 (81.3-96.3)*	ELISA	<25 kg/m^2**	**Normal weight**
38	Laura, 2013	Prospective cohort study	29	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	77.8 (63.1-96.0)*	ELISA	25.0-29.9 kg/m^2**	**Overweight**
38	Laura, 2013	Prospective cohort study	36	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	69.9 (59.0-82.9)*	ELISA	≥30 kg/m^2**	**Obese**
41	Zhang, 2008	CCS	114	Tacoma, Washington. 47.2529° N, 122.4443° W	September 2002 to October 2004	~16 wk	**2nd trimester**	75.2 ± 24.2	ELISA	23.3 ±3.8**	**Normal weight**
42	Zhou, 2014	Prospective study	370	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	41.4 ± 6.5	ECLIA	20.2 ±2.5**	**Normal weight**
42	Zhou, 2014	Prospective study	946	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	62.1 ± 6.9	ECLIA	20.4 ±2.5**	**Normal weight**

42	Zhou, 2014	Prospective study	637	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	89.7 ± 13.0	ECLIA	20.6 ±2.6**	**Normal weight**
43	Bartoszewicz, 2013	Cohort study	50	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 – – March 31) and summer (April 1 – September 30)	2nd trimester	**2nd trimester**	62.0 ±23.2	ECLIA	23.4 ± 3.1#	**Normal weight**
46	Karlsson, 2014	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	2nd trimester	**2nd trimester**	58.2 ± 18.3	CLIA	22.0 ± 1.4#	**Normal weight**
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	2nd trimester	**2nd trimester**	49.7 ± 18.9	CLIA	33.9 ± 3.3#	**Obese**
51	Boyle, 2016	Prospective cohort	1710	New Zealand 40.9006° S, 174.8860° E	2005-2008	15 wk	**2nd trimester**	72.9 ± 27.0	LC-MS/MS	24.8 ± 4.2	**Normal weight**
52	Eggemoen, 2017	Prospective cohort	719	Oslo, Norway 59.9139° N, 10.7522° E	May 2008 to March 2010	15.4 3.5	**2nd trimester**	50.0 ± 27.0	RIA	24·5±4·8**	**Normal weight**
Observational studies of vitamin D status in the second & third trimesters											
32	Parildar, 2013	CCS	78	Istanbul 41.0082° N, 28.9784° E	2009 to 2011	24-32 wk	**2nd &3rd trimester**	57.25± 25	ELISA	25.9± 4.4	**Obese**

53	Siddiqi, 2018	CS	50	Aligarh, India 27.8974° N, 78.0880° E	January 2014 to November 2015	24-28 wk	**2nd &3rd trimester**	80.8 ± 21.0	CLIA	23.9 ± 2.6	**Normal weight**
54	Tänczer, 2017	CCS	45	Budapest, Hungary 47.4979° N, 19.0402° E	January 1 2005 to December 31 2006	24-28 wk	**2nd &3rd trimester**	67.3 ± 24.5	CLIA	24.3±4.4	**Normal weight**
55	Zhao, 2017	Cohort	11012	Wuxi, China 31.4912° N, 120.3119° E	January 2011 to December 2013.	23-28 wk	**2nd & 3rd trimester**	37.7 ± 14.1	CLIA	21.4 ±2.6**	**Normal weight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
CS- cross section, CCS-case control study, N/A not available, wk- week gestational.
* n 38,40 studies vitamin D and BMI presented as mean (95% CI)
** Pre-pregnancy BMI
# BMI from first-trimester

Table 1, c: Observational studies of vitamin D status in the third trimester

N	First author, year	Study design	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
3	Murat Bakacak, 2015	CCS	40	Turkey, Kahramanmaras 37.7503° N, 36.9541° E	N/A	38.5 ± 1.43 wk	**3rd trimester**	59.2 ± 14.8	ECLIA	30.6 ± 3.72	**Obese**	
5	Abedi, 2013	CC	59	Ahvaz, Iran 31.3183° N, 48.6706° E	July to November 2012	38.8 ± 1.23 wk	**3rd trimester**	57.3 ± 28.4	ELISA	23.6 ± 3.5#	**Normal weight**	
9	Bodnar, 2007	Prospective cohort	219	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	67.3 (58.8, 77.0)	ELISA	<25 kg/m^2**	**Normal weight**	
9	Bodnar, 2007	Prospective cohort	84	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	61.2 (52.0, 72.0)*	ELISA	25–29.9 kg/m^2 **	**Overweight**	
9	Bodnar, 2007	Prospective cohort	81	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	60.2 (51.0, 71.2)*	ELISA	≥30 kg/m^2 **	**Obese**	
10	Crozier, 2012	Prospective cohort study	969	Southampton, UK, 50.9097° N, 1.4044° W	April 1998 to December 2002.	34 wk	**3rd trimester**	68.4 ± 32.4	RIA	25.4 ±4.7**	**Overweight**	

11	Brembeck, 2013	CS	95	Gothenburg, Sweden 57.7089° N, 11.9746° E	July 2008 to July 2011	35–37 wk	**3rd trimester**	47·4 ± 18·1	CLIA	22·5 **	**Normal weight**
12	Dror, 2011	CS	206	Oakland, USA 37.8044° N, 122.2711° W	December 2006 to January 2008	1 month before their due date.	**3rd trimester**	74.2 ± 34.0	RIA	32.1 ±8.7**	**Obese**
14	El Rifai, 2014	CS	135	Cairo, Egypt 30.0273° N, 31.2086° E	September 2012 to May 2013	Immediately before delivery 38.6 ± 5.0	**3rd trimester**	81.5 ± 53.5	ELISA	31.8 ±5.0	**Obese**
16	Hanieh, 2015	Prospective cohort study	891	Ha Nam province in northern Vietnam 20.5835° N, 105.9230° E	September 2010 to January 2012	32 Wk	**3rd trimester**	70.6 ± 22.2	N/A	19.9 ± 2.0#	**Normal weight**
19	Godang, 2014	Prospective cohort study	202	Norway, Oslo 59.9139° N, 10.7522° E	2001 to 2008	30–32 wk	**3rd trimester**	45.0 ± 17.0	RIA	26.6 ± 3.8	**Overweight**
20	Gunduz, 2016	CS	92	Ankara, Turkey 39.9334° N, 32.8597° E	January 1, 2013 and July 1, 2013	36 wk	**3rd trimester**	57.2 ± 40.5	HPLC	24.2 ±3.2**	**Normal weight**

22	McManus, 2014	CCS	37	London, Ontario, Canada 49°N.	2008 to 2011 recruitment during winter months of November–March to minimize sunlight influences.	31 .4± 3. 6 wk	**3rd trimester**	93.2 ± 19. 2	RIA	27.2 ±7.2**	**Overweight**
24	Karras, 2013	CS	60	Thessaloniki, Greece 40.6401° N, 22.9444° E	January 2011 until December 2011	30-60 minutes before delivery	**3rd trimester**	44.7 ± 33.0	LC-MS/MS	22.2 ±3.3**	**Normal weight**
26	Josefson, 2013	CS	38	Chicago, 41° N	N/A	39.8± 1.1 wk	**3rd trimester**	n= 33 115.12	HPLC	22.0 ±1.8**	**Normal weight**
26	Josefson, 2013	CS	23	Chicago 41° N	N/A	39.8 ± 1.0 wk	**3rd trimester**	n= 15 124.6	HPLC	35.5 ±4.1**	**Obese**
27	Moon, 2015	Prospective cohort study	1753	Southampton, United Kingdom 50.98° N	April 1998 to October 2002	34 wk	**3rd trimester**	64.8 ± 30.4	RIA	25.2 ±4.6**	**Overweight**
33	Park, 2014	Prospective study	500	Seoul, Korea 37.5665° N, 126.9780° E	May 2011 to July 2012	32-34 wk	**3rd trimester**	48.0 ± 24.8	ECLIA	25.8 ± 3.0	**Overweight**
40	Bärebring, 2016	Cohort	1829	South-western Sweden 57-58°N	2013 (2 September–8 November) and 2014 (24 February–13 June)	33.4 ± 1.9	**3rd trimester**	74.7 ± 34.4	LC-MS/MS	24.5 ± 4.2#	**Normal weight**

43	Bartoszewicz, 2013	Cohort study	50	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 – – March 31) and summer (April 1 – September 30)	3rd trimester	**3rd trimester**	62.7 ± 26.0	ECLIA	23.4 ± 3.1#	**Normal weight**
44	Robinson, 2013	CCS	40	South Carolina 33.8361° N, 81.1637° W	2007 to 2011	29 ± 1.17 wk	**3rd trimester**	91.5 ± 27.4	RIA	29.2 ±2.6**	**Overweight**
45	Ullah, 2013	CCS	76	Bangladesh 23.6850° N, 90.3563° E	N/A	1week before estimated date for delivery	**3rd trimester**	62.1 ± 2.5	ECLIA	18.9 ± .02	**Normal weight**
46	Karlsson, 2015	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	3rd trimester	**3rd trimester**	51.7 ± 18.3	CLIA	22.0 ± 1.4#	**Normal weight**
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	3rd trimester	**3rd trimester**	47.7 ± 18.3	CLIA	33.9 ± 3.3#	**Obese**
56	Karras, 2018	CS	70	Thessaloniki, Greece 40.6401° N, 22.9444° E	April 2014 until October 2015	38.8 ± 0.2	**3rd trimester**	45.8 ± 3.0	RIA	29.6 ± 0.7	**Overweight**
57	Simões-Wüst, 2017	Prospective cohort study	767	Netherlands, Maastricht 50.8514° N, 5.6910° E	October 2000 until December 2002	34–36 wk	**3rd trimester**	44·4 ± 18·2	ELISA	23·8±3·9**	**Normal weight**
58	Ong, 2017	Prospective cohort	910	Singapore (1°22’ N)	N/A	26-28 wk	**3rd trimester**	81.3 ± 27.2	LC-MS/MS	26.1 ± 4.3	**Overweight**

52	Eggemoen, 2017	Prospective cohort	719	Oslo, Norway 59.9139° N, 10.7522° E	May 2008 to March 2010	28.8 1.4	**3rd trimester**	59.0 ± 29.0	RIA	24·5±4·8**	**Normal weight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by $height^2$ (m^2) (WHO).
CS- cross section, CCS-case control study, N/A not available, wk- week gestational.
** Pre-pregnancy BMI
# BMI from first-trimester

Table 1, d: Observational studies of cord blood vitamin D status

N	First author, year	Study design	Sample size (n)	Geographic location	Time (between)	25(OH)D nmol/L Mean ± SD	Vitamin D method	Maternal BMI (kg/m^2) Mean ± SD (or range)	BMI categories
9	Bodnar, 2007	Prospective cohort	216	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	56.2 (49.7, 63.6) *	ELISA	<25 kg/m^2 **	**Normal weight**
9	Bodnar, 2007	Prospective cohort	80	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	53.8 (46.2, 62.8)*	ELISA	25–29.9 kg/m^2 **	**Overweight**
9	Bodnar, 2007	Prospective cohort	70	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	49.9 (42.8, 58.2)*	ELISA	≥30 kg/m^2 **	**Obese**
12	Dror, 2011	CS observation study	199	Oakland, USA 37.8044° N, 122.2711° W	December 2006 to January 2008	43.7 ± 23	RIA	32.1 ± 8.7 **	**Obese**
14	El Rifai, 2014	CS	135	Cairo University, Egypt 30.0273° N, 31.2086° E	September 2012 to May 2013	41.7 ± 25	ELISA	31.8 ± 5.0#	**Obese**
19	Godang, 2014	Prospective cohort	202	Norway, Oslo 59.9139° N, 10.7522° E	2001 to 2008	31 ± 18	RIA	26.6 ± 3.8#	**Overweight**
22	McManus, 2014	CCS	37	London, Ontario, Canada latitude of 49°N.	2008 to 2011 recruitment during winter months of November–March to minimize sunlight influences.	64.8 ± 11.5	RIA	27.2± 7.2**	**Overweight**

24	Karras, 2013	CS	60	Thessaloniki, Greece 40.6401° N, 22.9444° E	January 2011 until December 2011	39.7 ± 34	LC-MS/MS	22.4 ±4.3**	**Normal weight**
26	Josefson, 2013	CS	35	Chicago, which is at latitude 41° North	Summer births were defined as those between June and October	68.6	HPLC	22.0+1.8**	**Normal weight**
26	Josefson, 2013	CS	23	Chicago, which is at latitude 41° North	Summer births were defined as those between June and October	52	HPLC	35.5+4.1**	**Obese**
47	Riaz, 2018	Prospective study	121	Karachi, Pakistan24.8607° N, 67.0011° E	January 2012 to May 2014	47.3 ± 35.8	ELISA	22.4 ± 4.6	**Normal weight**
56	Karras, 2018	CS	70	Thessaloniki, Greece 40.6401° N, 22.9444° E	April 2014 until October 2015	40.8 ± 2.5	RIA	29.6 ± 0.7	**Overweight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
CS- cross section, CCS-case control study, N/A not available, wk- week gestational.
* n 9 study vitamin D presented as mean (95% CI)
** Pre-pregnancy BMI
# BMI from first-trimester

Table 2, a: Prospective studies

N	First author, year	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
27	Moon, 2015	1753	Southampton, UK 50.9097° N, 1.4044° W	April 1998 to October 2002	11 wk	**1st trimester**	62.7 ± 25.9	LC-MS/MS	25.2±4.6**	**Overweight**	
		1753			34 wk	**3rd trimester**	64.8 ± 30.4	RIA	25.2±4.6**	**Overweight**	
33	Park, 2014	500	Seoul, Korea 37.5665° N, 126.9780° E	May 2011 to July 2012	12-14 wk	**1st trimester**	32.0 ± 14.5	ECLIA	21.2 ± 2.8	**Normal weight**	
		500			20-22 wk	**2nd trimester**	46.5 ± 23.6	ECLIA	22.6 ± 2.8	**Normal weight**	
		500			32-34 wk	**3rd trimester**	48.0 ± 24.8	ECLIA	25.8 ± 3.0	**Overweight**	
34	Savvidou, 2012	796	London, UK 51.5074° N, 0.1278° W	01-Mar-06	11–13 wk	**1st trimester**	47.6 ± 11.7	LC-MS/MS	24.0 ± 1.4	**Normal weight**	Women had vaginal delivery
34	Savvidou, 2012	111	London, UK 51.5074° N, 0.1278° W	01-Mar-06	11–13 wk	**1st trimester**	45.0 ± 14.2	LC-MS/MS	25.9 ± 1.8	**Overweight**	Women had emergency C- section
34	Savvidou, 2012	88	London, UK 51.5074° N, 0.1278° W	01-Mar-06	11–13 wk	**1st trimester**	55.9 ± 14.6	LC-MS/MS	26.1 ± 1.6	**Overweight**	Women had Elective C- section

47	Riaz, 2018	301	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.78 ± 2.30 wk	**1st trimester**	40.9 ± 32.3	ELISA	22.4 ± 4.6	**Normal weight**	All women
47	Riaz, 2018	63	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.54 ± 2.40 wk	**1st trimester**	43.7 ± 30.7	ELISA	17.2 ± 1.0	**Normal weight**	
47	Riaz, 2018	130	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.79 ± 2.25 wk	**1st trimester**	38.3 ± 29.4	ELISA	20.9 ± 1.3	**Normal weight**	
47	Riaz, 2018	108	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.92 ± 2.30 wk	**1st trimester**	42.4 ± 36.3	ELISA	27.3 ± 4.0	**Overweight**	
		121			Cord	**Cord**	47.3 ± 35.8	ELISA	22.4 ± 4.6	**Normal weight**	All women
9	Bodnar, 2007	223	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	62.8(55.0, 70.4)*	ELISA	<25 kg/m^2**	**Normal weight**	
9	Bodnar, 2007	87	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	58.6 (51.5, 66.8)*	ELISA	25–29.9 kg/m^2**	**Overweight**	
9	Bodnar, 2007	82	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	55.9 (48.7, 64.2) *	ELISA	≥30 kg/m^2 **	**Obese**	

		219			37–42 wk	**3rd trimester**	67.3 (58.8, 77.0)	ELISA	<25 kg/m^2**	**Normal weight**
		84			37–42 wk	**3rd trimester**	61.2 (52.0, 72.0)*	ELISA	25–29.9 kg/m^2 **	**Overweight**
		81			37–42 wk	**3rd trimester**	60.2 (51.0, 71.2)*	ELISA	≥30 kg/m^2 **	**Obese**
		216			Cord	**Cord**	56.2 (49.7, 63.6) *	ELISA	<25 kg/m^2 **	**Normal weight**
		80			Cord	**Cord**	53.8 (46.2, 62.8)*	ELISA	25–29.9 kg/m^2 **	**Overweight**
		70			Cord	**Cord**	49.9 (42.8, 58.2)*	ELISA	≥30 kg/m^2 **	**Obese**
49	Valkama, 2018	43	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	74 ± 23	N/A	<25 kg/m^2	**Normal weight**
49	Valkama, 2018	34	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	73 ± 32	N/A	25-<30 kg/m^2	**Overweight**
49	Valkama, 2018	84	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	64 ± 24	N/A	30-<35 kg/m^2	**Obese**
49	Valkama, 2018	58	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	57 ± 22	N/A	≥35 kg/m^2	**Obese class 2**
7	Aghajafari, 2016	537	Alberta, Canada 53.9333° N, 116.5765° W	March 2009 to July 2012	Second trimester	**2nd trimester**	95.3 ± 25.0	LC-MS/MS	26.0 ± 5.0	**Overweight**

18	Huang, 2013	658	Washington. Seattle, Tacoma 47.6062° N, 122.3321° W	1996 to 2008	At or prior to 20 wk	**2nd trimester**	72.7 ± 21.0	LC-MS/MS	23.8±5.4**	**Normal weight**
21	Hrudey, 2016	1882	Amsterdam 52.3702° N, 4.8952° E	January 2003 to March 2004,	16.1 wk	**2nd trimester**	60.4 ± 0.7	ELISA	22.9 ± 3.7	**Normal weight**
36	Singla, 2015	304	North India 20.5937° N, 78.9629° E	2010 to 2013	12–16 wk	**2nd trimester**	20.9 ± 4.5	ELISA	21.8 ± 3.4	**Normal weight**
38	Laura, 2013	64	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	88.5 (81.3-96.3)*	ELISA	<25 kg/m^2**	**Normal weight**
38	Laura, 2013	29	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	77.8 (63.1-96.0)*	ELISA	25.0-29.9 kg/m^2**	**Overweight**
38	Laura, 2013	36	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	69.9 (59.0-82.9)*	ELISA	≥30 kg/m^2**	**Obese**
42	Zhou, 2014	370	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	41.4 ± 6.5	ECLIA	20.2±2.5**	**Normal weight**

42	Zhou, 2014	946	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	62.1 ± 6.9	ECLIA	20.4±2.5**	**Normal weight**
42	Zhou, 2014	637	Guangzhou, China 23.1291° N, 113.2644° E	September 2010 to August 2011, which followed up to 2012	16–20 wk	**2nd trimester**	89.7 ± 13.0	ECLIA	20.6±2.6**	**Normal weight**
51	Boyle, 2016	1710	New Zealand 40.9006° S, 174.8860° E	2005-2008	15 wk	**2nd trimester**	72.9 ± 27.0	LC-MS/MS	24.8 ± 4.2	**Normal weight**
52	Eggemoen, 2017	719	Oslo, Norway 59.9139° N, 10.7522° E	May 2008 to March 2010	15.4 3.5	**2nd trimester**	50.0 ± 27.0	RIA	24·5±4·8**	**Normal weight**
		719			28.8 1.4	**3rd trimester**	59.0 ± 29.0	RIA	24·5±4·8**	**Normal weight**
10	Crozier, 2012	969	Southampton, UK, 50.9097° N, 1.4044° W	April 1998 to December 2002.	34 wk	**3rd trimester**	68.4 ± 32.4	RIA	25.4±4.7**	**Overweight**
16	Hanieh, 2015	891	Ha Nam province in northern Vietnam 20.5835° N, 105.9230° E	September 2010 to January 2012	32 Wk	**3rd trimester**	70.6 ± 22.2	N/A	19.9 ± 2.0#	**Normal weight**

57	Simões-Wüst, 2017	767	Netherlands, Maastricht 50.8514° N, 5.6910° E	October 2000 until December 2002	34–36 wk	**3rd trimester**	44·4 ± 18·2	ELISA	23·8±3·9**	**Normal weight**
58	Ong, 2017	910	Singapore 1°22' N	N/A	26-28 wk	**3rd trimester**	81.3 ± 27.2	LC-MS/MS	26.1 ± 4.3	**Overweight**
19	Godang, 2014	202	Norway, Oslo 59.9139° N, 10.7522° E	2001 to 2008	30–32 wk	**3rd trimester**	45.0 ± 17.0	RIA	26.6 ± 3.8	**Overweight**
		202			Cord	**Cord**	31 ± 18	RIA	26.6 ± 3.8#	**Overweight**
46	Karlsson, 2014	80	Western Sweden region 57° N	April 2009 to November 2012	11.9 ± 1.0 wk	**1st trimester**	64.2 ± 18.3	CLIA	22.0 ± 1.4	**Normal weight**
46	Karlsson, 2014	25	Western Sweden region 57° N	April 2009 to November 2012	11.5 ± 1.5 wk	**1st trimester**	49.7 ± 11.5	CLIA	33.9 ± 3.3	**Obese**
		80			2nd trimester	**2nd trimester**	58.2 ± 18.3	CLIA	22.0 ± 1.4#	**Normal weight**
		25			2nd trimester	**2nd trimester**	49.7 ± 18.9	CLIA	33.9 ± 3.3#	**Obese**
		80			3rd trimester	**3rd trimester**	51.7 ± 18.3	CLIA	22.0 ± 1.4#	**Normal weight**
		25			3rd trimester	**3rd trimester**	47.7 ± 18.3	CLIA	33.9 ±3.3#	**Obese**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
N/A not available, wk- week gestational.
* n 9 study vitamin D presented as mean (95% CI)
** Pre-pregnancy BMI
# BMI from first-trimester

Table 2, b: Retrospective analysis

N	First author, year	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
23	McAree, 2013	227	North West London 51.5074° N, 0.1278° W	April 2008 and March 2009	At the initial booking visit	**1st trimester**	39.0 ± 12.7	LC-MS	<30 kg/m^2	**Normal weight & overweight**	
23	McAree, 2013	43	North West London 51.5074° N, 0.1278° W	April 2008 and March 2009	At the initial booking visit	**1st trimester**	29.0 ± 10.9	LC-MS	≥30 kg/m^2	**Obese**	

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).

Table 2, c: Cohort studies

N	First author, year	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
39	Gerrit van den Berg, 2013	372	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	49.8 ± 23.7	ELISA	BMI >25 kg/m^2 **	**Overweight**	Low education
39	Gerrit van den Berg, 2013	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	58.3± 24.1	ELISA	BMI >25 kg/m^2 **	**Overweight**	Mid education
39	Gerrit van den Berg, 2013	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	62.6 ± 27.5	ELISA	BMI >25 kg/m^2 **	**Overweight**	High education
39	Gerrit van den Berg, 2013	1902	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	63.6 ± 39.8	ELISA	BMI <25 kg/m^2 **	**Normal weight**	Low education
39	Gerrit van den Berg, 2013	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	71.5± 28.4	ELISA	BMI <25 kg/m^2 **	**Normal weight**	Mid education
39	Gerrit van den Berg, 2013	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	69.1± 27.0	ELISA	BMI <25 kg/m^2 **	**Normal weight**	High education
43	Bartoszewicz, 2013	50	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 — March 31) and summer (April 1 – September 30)	1st trimester	**1st trimester**	57.7 ± 22.2	ECLIA	23.4 ±3.1	**Normal weight**	

		50			2nd trimester	**2nd trimester**	62.0 ±23.2	ECLIA	23.4 ± 3.1#	**Normal weight**
		50			3rd trimester	**3rd trimester**	62.7 ± 26.0	ECLIA	23.4 ± 3.1#	**Normal weight**
15	Davies-Tuck, 2015	1550	Monash, Australia 37.9016° S, 145.1155° E	July 2009 to June 2010	13.7± 3.3 wk	**2nd trimester**	71.0 ± 47.9	ECLIA	31.2 ± 13.5	**Obese**
55	Zhao, 2017	11012	Wuxi, China 31.4912° N, 120.3119° E	01/01/2011to December 2013.	23-28 wk	**2nd & 3rd trimester**	37.7 ± 14.1	CLIA	21.4±2.6**	**Normal weight**
40	Bärebring, 2016	1829	South-western Sweden 57-58°N	2013 (2 September–8 November) and 2014 (24 February–13 June).	10.8 ± 2.0 wk	**1st trimester**	64.5 ± 24.5	LC-MS/MS	24.5 ± 4.2	**Normal weight**
		1829			33.4 ± 1.9	**3rd trimester**	74.7 ± 34.4	LC-MS/MS	24.5 ± 4.2#	**Normal weight**
50	Benjamin Neelon, 2018	476	Durham, North Carolina 35.9940° N, 78.8986° W	July 2009 to December 2011	13.2 ± 5.5 wk	**1st & 2nd trimester**	41.1 ± 14.2	IDS	27.4±7.0**	**Overweight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
N/A not available, wk- week gestational.
** Pre-pregnancy BMI
# BMI from first-trimester

Table 2, d: Case-control studies

N	First author, year	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
25	Makgoba, 2011	158	Glasgow, UK 55.8642° N, 4.2518° W	N/A	~12.5 wk	**1st trimester**	47.6 ± 26.7	LC-MS/MS	25.2 ± 4.0	**Overweight**	
31	Powe, 2011	131	Boston, USA 42.3601° N, 71.0589° W	N/A	11.6 ± 3.0 wk	**1st trimester**	72.0 ± 2.0	LC-MS/MS	24.4 ± 4.5	**Normal weight**	
48	Woolcott, 2016	1002	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	54.6 ± 16.8	CLIA	<25 kg/m^2**	**Normal weight**	
48	Woolcott, 2016	311	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	51.0 ± 15.6	CLIA	25-<30 kg/m^2**	**Overweight**	
48	Woolcott, 2016	124	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	48.2 ± 16.5	CLIA	30-<35 kg/m^2**	**Obese**	
48	Woolcott, 2016	84	Quebec 52° 56' N 73° 32' W Halifax 44° 38' N 63° 34' W Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	45.7 ± 15.4	CLIA	≥35 kg/m^2**	**Obese class 2**	

13	Daglar, 2016	30	Ankara, Turkey 39.9334° N, 32.8597° E	01/03/2014 to May 2014	19.4 ± 4.6 wk	**2nd trimester**	22.7 ± 18.2	ELISA	26.1 ± 5.2	**Overweight**
41	Zhang, 2008	114	Tacoma, Washington. 47.2529° N, 122.4443° W	September 2002 to 01/10/2004	..16 wk	**2nd trimester**	75.2 ± 24.2	ELISA	23.3±3.8**	**Normal weight**
54	Tänczer, 2017	45	Budapest, Hungary 47.4979° N, 19.0402° E	January 1 2005 to December 31 2006	24-28 wk	**2nd &3rd trimester**	67.3 ± 24.5	CLIA	24.3±4.4	**Normal weight**
32	Parildar, 2013	78	Istanbul 41.0082° N, 28.9784° E	2009 to 2011	24-32 wk	**2nd &3rd trimester**	57.25± 25	ELISA	25.9± 4.4	**Obese**
3	Murat Bakacak, 2015	40	Turkey, Kahramanmaras 37.7503° N, 36.9541° E	N/A	38.5 ± 1.43 wk	**3rd trimester**	59.2 ± 14.8	ECLIA	30.6 ± 3.72	**Obese**
5	Abedi, 2013	59	Ahvaz, Iran 31.3183° N, 48.6706° E	July to November 2012	38.8 ± 1.23 wk	**3rd trimester**	57.3 ± 28.4	ELISA	23.6 ± 3.5#	**Normal weight**
22	McManus, 2014	37	London, Ontario, Canada 49°N.	2008 to 2011 recruitment during winter months	31 .4± 3. 6 wk	**3rd trimester**	93.2 ± 19. 2	RIA	27.2±7.2**	**Overweight**
		37			Cord	**Cord**	64.8 ± 11.5	RIA	27.2±7.2**	**Overweight**

44	Robinson, 2013	40	South Carolina 33.8361° N, 81.1637° W	2007 to 2011	29 ± 1.17 wk	**3rd trimester**	91.5 ± 27.4	RIA	29.2±2.6**	**Overweight**
45	Ullah, 2013	76	Bangladesh 23.6850° N, 90.3563° E	N/A	1week before estimated date for delivery	**3rd trimester**	62.1 ± 2.5	ECLIA	18.9 ± .02	**Normal weight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by $height^2$ (m^2) (WHO).
N/A not available, wk- week gestational.
** Pre-pregnancy BMI
# BMI from first-trimester

Table 2, e: Cross sectional studies

N	First author, year	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
8	Bukhary, 2016	396	West Peninsular Malaysia, Selangor 3.0738° N, 101.5183° E	1st January until end of April 2014	First trimester	**1st trimester**	27.7 ± 4.9	ECLIA	22.9±1.8**	**Normal weight**	
30	Perez-Lopez, 2011	502	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	67.8 ± 8.5	ECLIA	N/A	**N/A**	
30	Perez-Lopez, 2011	307	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	70.2 ± 7.9	ECLIA	<25 kg/m^2	**Normal weight**	
30	Perez-Lopez, 2011	132	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	64.1 ± 9.0	ECLIA	25-30 kg/m^2	**Overweight**	
30	Perez-Lopez, 2011	63	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	60.6 ±10.0	ECLIA	≥ 30 kg/m^2	**Obese**	
4	Al-Ajlan, 2015	515	Riyadh, Saudi Arabia 24.7136° N, 46.6753° E	N/A	11.2 ± 2.7wk	**1st trimester**	23.4 ± 15.7	ECLIA	28.4 ± 6.2	**Overweight**	
2	Andersen, 2013	36	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	59.7	LC-MS/MS	<18.5 kg/m^2	**Under weight**	

2	Andersen, 2013	740	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	66.6	LC-MS/MS	18.5–24.9 kg/m^2	**Normal weight**
2	Andersen, 2013	300	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	62.6	LC-MS/MS	25–29.9 kg/m^2	**Overweight**
2	Andersen, 2013	81	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	56.7	LC-MS/MS	30–34.9 kg/m^2	**Obese**
2	Andersen, 2013	37	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	52.1	LC-MS/MS	>35 kg/m^2	**Obese class 2**
1	Allen, 2013	98	Western Australia 27.6728° S, 121.6283° E	1989 to 1991	18 wk	**2nd trimester**	55.0 ± 17.1	ELISA	22.6 ± 4.4	**Normal weight**
1	Allen, 2013	428	Western Australia 27.6728° S, 121.6283° E	1989 to 1991	18 wk	**2nd trimester**	58.6 ± 16.3	ELISA	21.8 ± 3.7	**Normal weight**
6	Mehmet Bal, 2016	50	İzmir, Turkey 38.4237° N, 27.1428° E	All blood sampling	24-28 wk	**2nd trimester**	63.5 ± 23.2	LC-MS/MS	22.8 ± 1.6	**Normal weight**
28	Ozias, 2014	193	Kansas, US 39.0119° N, 98.4842° W	January 2006 to November 2009	14 ± 4 wk	**2nd trimester**	56.7 ± 33.2	EIA	27.0±5.0**	**Overweight**

29	Perampalam, 2011	100	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	61.3 ± 23.4	ECLIA	27.3 ± 5.7	**Overweight**	All women
29	Perampalam, 2011	33	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	65.9 ± 28.8	ECLIA	16.0 - 24.9 kg/m^2	**Normal weight**	
29	Perampalam, 2011	44	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	61.6 ± 21.2	ECLIA	25.0- 29.9 kg/m^2	**Overweight**	
29	Perampalam, 2011	15	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	53.4 ± 17.2	ECLIA	30.0- 34.9 kg/m^2	**Obese**	
29	Perampalam, 2011	8	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	60.7 ± 21.9	ECLIA	≥35.0 kg/m^2	**Obese class 2**	
29	Perampalam, 2011	101	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	69.5 ± 26.9	RIA	28.3 ± 5.8	**Overweight**	All women
29	Perampalam, 2011	36	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	62.8 ± 24.3	RIA	16.0- 24.9 kg/m^2	**Normal weight**	
29	Perampalam, 2011	29	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	58.9 ± 30.1	RIA	25.0- 29.9 kg/m^2	**Overweight**	

29	Perampalam, 2011	21	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49.0 ± 17.4	RIA	30.0- 34.9 kg/m^2	**Obese**	
29	Perampalam, 2011	13	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49.1 ± 25.0	RIA	≥35.0 kg/m^2	**Obese class 2**	
35	Shiraishi, 2014	284	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	17.0 ± 11.7	ECLIA	20.4±2.4**	**Normal weight**	All women
35	Shiraishi, 2014	157	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	25.7 ± 12.7	ECLIA	20.3±2.3**	**Normal weight**	During summer
35	Shiraishi, 2014	127	Tokyo, Japan 35.6895° N, 139.6917° E	June 2010 to July 2011	19- 23 wk	**2nd trimester**	23.0 ± 10.5	ECLIA	20.6±2.6**	**Normal weight**	During winter
37	Tao, 2012	1695	Shanghai, China 31.2304° N, 121.4737° E	01/07/2008 to 30 June 2009	21.0 ± 6.2 wk	**2nd trimester**	43.9 ± 28.6	ECLIA	22.5 ± 3.1	**Normal weight**	
17	Huang, 2014	498	Seattle, Washington. 47.6062° N, 122.3321° W	April 2009 to December 2010	At or prior to 20 wk	**2nd trimester**	86.0 ± 21.7	LC-MS/MS	23.5±4.7**	**Normal weight**	
53	Siddiqi, 2018	50	Aligarh, India 27.8974° N, 78.0880° E	January 2014 to 01/11/2015	24-28 wk	**2nd &3rd trimester**	80.8 ± 21.0	CLIA	23.9 ± 2.6	**Normal weight**	
11	Brembeck, 2013	95	Gothenburg, Sweden 57.7089° N, 11.9746° E	July 2008 to July 2011	35–37 wk	**3rd trimester**	47·4 ± 18·1	CLIA	22·5 **	**Normal weight**	

12	Dror, 2011	206	Oakland, USA 37.8044° N, 122.2711° W	December 2006 to January 2008	1 month before their due date.	**3rd trimester**	74.2 ± 34.0	RIA	32.1±8.7**	**Obese**
		199			Cord	**Cord**	43.7 ± 23	RIA	32.1±8.7**	**Obese**
14	El Rifai, 2014	135	Cairo, Egypt 30.0273° N, 31.2086° E	September 2012 to May 2013	Immediately before delivery 38.6 ± 5.0	**3rd trimester**	81.5 ± 53.5	ELISA	31.8 ±5.0	**Obese**
		135			Cord	**Cord**	41.7 ± 25	ELISA	31.8 ±5.0	**Obese**
20	Gunduz, 2016	92	Ankara, Turkey 39.9334° N, 32.8597° E	January 1, 2013 and July 1, 2013	36 wk	**3rd trimester**	57.2 ± 40.5	HPLC	24.2±3.2**	**Normal weight**
26	Josefson, 2013	38	Chicago, 41° N	N/A	39.8± 1.1 wk	**3rd trimester**	n= 33 115.12	HPLC	22.0±1.8**	**Normal weight**
		35		Summer births were defined as those between June and October	Cord	**Cord**	68.6	HPLC	22.0±1.8**	**Normal weight**
26	Josefson, 2013	23	Chicago 41° N	N/A	39.8 ± 1.0 wk	**3rd trimester**	n= 15 124.6	HPLC	35.5±4.1**	**Obese**
		38		Summer births were defined as those between June and October	Cord	**Cord**	52	HPLC	35.5±4.1**	**Obese**

56	Karras, 2018	70	Thessaloniki, Greece 40.6401° N, 22.9444° E	April 2014 until October 2015	38.8 ± 0.2	**3rd trimester**	45.8 ± 3.0	RIA	29.6 ± 0.7	**Overweight**
		70			Cord	**Cord**	40.8 ± 2.5	RIA	29.6 ± 0.7	**Overweight**
24	Karras, 2013	60	Thessaloniki, Greece 40.6401° N, 22.9444° E	April 2009 to November 2012	30-60 minutes before delivery	**3rd trimester**	44.7 ± 33.0	LC-MS/MS	22.2±3.3**	**Normal weight**
24		60			Cord	**Cord**	39.7 ± 34	LC-MS/MS	22.4±4.3**	**Normal weight**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
N/A not available, wk- week gestational.
** Pre-pregnancy BMI
# BMI from first-trimester

Table 3: Randomised clinical studies of vitamin D supplementation during pregnancy

N	First author, year	Study design	Participants	Sample size	Geographic location	Time (between)	Supplement use	Dietary information µg/d	Gestation age Mean ±SD	25(OH)D nmol/L mean ± SD	Vitamin D method	BMI (kg/m2) mean ± SD
1	Asemi, 2013	RCT	Pregnant women, with 10 µg vitamin D_3 supplements	24	Kashan, Iran 33.9850° N, 51.4100° E	March 2012 to September 2012	10 µg/d vitamin D_3 for 9 wk	Run-in period 2.8 ± 0.9 throughout the study 2.9 ± 0.9	25 wk	baseline 44.5± 3.25	HPLC	BMI baseline 26.8 ± 3.9
									34wk	9 weeks 53.7± 4.5		BMI at end 28.0 ± 3.9
1	Asemi, 2013	RCT	Pregnant women, with placebo	24	Kashan, Iran 33.9850° N, 51.4100° E	March 2012 to September 2012	Placebo for 9 wk	Run-in period 2.8 ± 0.7 throughout the study 2.9 ± 0.8	25WK	baseline 14.5 ± 1.2	HPLC	BMI baseline, 27.4 ± 4.0
									34WK	9 weeks 13.3 ± 1.1		BMI at end 28.8 ± 3.7
2	Callaghan, 2018	RCT	Pregnant women, with 20 µg vitamin D_3 supplements	48	Cork, Ireland 51.8969° N, 8.4863° W	November 2014 until April 2016	20 µg vitamin D_3 from 14 wk until delivery	Form diet and supplement 11.4 ± 5.0	14 wk	58.0 ± 22.9	LC-MS/MS	24.5 ± 3.1
				44					24 wk	92.8 ± 22.1		
				44					36 wk	100.6 ± 23.3		
				32					cord	50.5 ± 15.1		
2	Callaghan, 2018	RCT	Pregnant women, with 10 µg vitamin D_3 supplements	48	Cork, Ireland 51.8969° N, 8.4863° W	November 2014 until April 2016	10 µg vitamin D_3 from 14 wk until delivery	Form diet and supplement 10.5 ± 5.4	14 wk	49.6 ± 19.6	LC-MS/MS	26.8 ± 5.1
				40					24 wk	81.9 ± 27.8		
				37					36 wk	96.0 ± 29.2		
				33					cord	44.1 ± 14.6		

2	Callaghan, 2018	RCT	Pregnant women, with placebo	48	Cork, Ireland 51.8969° N, 8.4863° W	November 2014 until April 2016	0 µg vitamin D3 from 14 wk until delivery	Form diet and supplement 10.6 ± 5.2	14 wk	57.2 ± 24.5	LC-MS/MS	25.7 ± 4.3
				43					24 wk	63.6 ± 25.0		
				40					36 wk	71.4 ± 24.3		
				32					cord	39.2 ± 16.3		

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
RCT-randomised control trail.

Table 4: Observational studies vitamin D status through pregnancy and divided the cohort based on BMI Categories

N	First author, year	Study design	Sample size (n)	Geographic location	Time (between)	Gestation age Mean ± SD (or range)	Gestation age category	25(OH)D nmol/L Mean ± SD	Vitamin D method	BMI (kg/m^2) Mean ± SD (or range)	BMI categories	Note
2	Andersen, 2013	CS	36	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012	8-16 wk	**1st & 2nd trimester**	59.7	LC-MS/MS	<18.5 kg/m^{2}**	**Under weight**	
2	Andersen, 2013	CS	740	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012	8-16 wk	**1st & 2nd trimester**	66.6	LC-MS/MS	18.5–24.9 kg/m^{2}**	**Normal weight**	
2	Andersen, 2013	CS	300	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012.	8-16 wk	**1st & 2nd trimester**	62.6	LC-MS/MS	25–29.9 kg/m^{2}**	**Overweight**	
2	Andersen, 2013	CS	81	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012	8-16 wk	**1st & 2nd trimester**	56.7	LC-MS/MS	30–34.9 kg/m^{2}**	**Obese**	
2	Andersen, 2013	CS	37	Odense, Denmark 55.4038° N, 10.4024° E	January 1st 2010 to April 2012	8-16 wk	**1st & 2nd trimester**	52.1	LC-MS/MS	> 35 kg/m^{2}**	**Obese class 2**	
9	Bodnar, 2007	Prospective cohort	223	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	62.8(55.0, 70.4)*	ELISA	<25 kg/m^{2}**	**Normal weight**	
9	Bodnar, 2007	Prospective cohort	87	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	58.6 (51.5, 66.8)*	ELISA	25–29.9 kg/m^{2}**	**Overweight**	

9	Bodnar, 2007	Prospective cohort	82	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	4–22 wk	**1st & 2nd trimester**	55.9 (48.7, 64.2) *	ELISA	≥30 kg/m^2**	**Obese**
9	Bodnar, 2007	Prospective cohort	219	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	67.3 (58.8, 77.0)*	ELISA	<25 kg/m^2**	**Normal weight**
9	Bodnar, 2007	Prospective cohort	84	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	61.2 (52.0, 72.0)*	ELISA	25–29.9 kg/m^2**	**Overweight**
9	Bodnar, 2007	Prospective cohort	81	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	37–42 wk	**3rd trimester**	60.2 (51.0, 71.2)*	ELISA	≥30 kg/m^2**	**Obese**
9	Bodnar, 2007	Prospective cohort	216	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	Cord	**Cord**	56.2 (49.7, 63.6)*	ELISA	<25 kg/m^2**	**Normal weight**
9	Bodnar, 2007	Prospective cohort	80	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	Cord	**Cord**	53.8 (46.2, 62.8)*	ELISA	25–29.9 kg/m^2**	**Overweight**
9	Bodnar, 2007	Prospective cohort	70	Pittsburgh, Magee 40.4406° N, 79.9959° W	1997 to 2001	Cord	**Cord**	49.9 (42.8, 58.2)*	ELISA	≥30 kg/m^2**	**Obese**
23	McAree, 2013	Retrospective analysis	227	North West London 51.5074° N, 0.1278° W	April 2008 to March 2009	At the initial booking visit	**1st trimester**	39 ± 12.7	LC-MS	<30 kg/m^2	**Normal weight & Overweight**

23	McAree, 2013	Retrospective analysis	43	North West London 51.5074° N, 0.1278° W	April 2008 to March 2009	At the initial booking visit	**1st trimester**	29 ± 10.9	LC-MS	≥30 kg/m^2	**obese**
26	Josefson, 2013	CS	38	Chicago, 41° N	N/A	39.8± 1.1 wk	**3rd trimester**	n= 33 115.12	HPLC	22.0±1.8**	**Normal weight**
26	Josefson, 2013	CS	23	Chicago 41° N	N/A	39.8 ±1.0 wk	**3rd trimester**	n= 15 124.6	HPLC	35.5±4.1**	**Obese**
26	Josefson, 2013	CS	35	Chicago, which is at latitude 41° North	Summer births were defined as those between June and October	Cord	**Cord**	68.6	HPLC	22.0+1.8**	**Normal weight**
26	Josefson, 2013	CS	23	Chicago, which is at latitude 41° North	Summer births were defined as those between June and October	Cord	**Cord**	52	HPLC	35.5+4.1**	**Obese**
29	Perampalam, 2011	CS	100	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	61.3 ± 23.4	ECLIA	27.3 ± 5.7	**Overweight** All women
29	Perampalam, 2011	CS	33	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	65.9 ± 28.8	ECLIA	16.0–24.9 kg/m^2	**Normal weight**
29	Perampalam, 2011	CS	44	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	61.6 ± 21.2	ECLIA	25.0–29.9 kg/m^2	**Overweight**

29	Perampalam, 2011	CS	15	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	53.4 ± 17.2	ECLIA	30.0–34.9 kg/m^2	**Obese**	
29	Perampalam, 2011	CS	8	Australia – Canberra 35.2809° S, 149.1300° E	August 2008 to October 2009	24-28 wk	**2nd trimester**	60.7 ± 21.9	ECLIA	≥35.0 kg/m^2	**Obese class 2**	
29	Perampalam, 2011	CS	101	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	69.5 ± 26.9	RIA	28.3 ± 5.8	**Overweight**	All women
29	Perampalam, 2011	CS	36	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	62.8 ± 24.3	RIA	16.0–24.9 kg/m^2	**Normal weight**	
29	Perampalam, 2011	CS	29	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	58.9 ± 30.1	RIA	25.0–29.9 kg/m^2	**Overweight**	
29	Perampalam, 2011	CS	21	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49 ± 17.4	RIA	30.0–34.9 kg/m^2	**Obese**	
29	Perampalam, 2011	CS	13	Campbell town 34.0650° S, 150.8142° E	September 2008 to February 2009	24-28 wk	**2nd trimester**	49.1± 25	RIA	≥35.0 kg/m^2	**Obese class 2**	
30	Perez-Lopez, 2011	CS	307	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	70.2 ± 7.9	ECLIA	<25 kg/m^2	**Normal weight**	

30	Perez-Lopez, 2011	CS	132	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	64.1 ± 9.0	ECLIA	25-30 kg/m^2	**Overweight**
30	Perez-Lopez, 2011	CS	63	Spanish Mediterranean sea coast 36° N, 2° W	May 2009 to April 2010	11 -14 wk	**1st trimester**	60.68±10.03	ECLIA	≥ 30 kg/m^2	**Obese**
38	Laura, 2013	Prospective cohort study	64	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	88.5 (81.3- 96.3)*	ELISA	<25 kg/m^{2}**	**Normal weight**
38	Laura, 2013	Prospective cohort study	29	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	77.8 (63.1-96.0)*	ELISA	25.0-29.9 kg/m^{2}**	**Overweight**
38	Laura, 2013	Prospective cohort study	36	Pittsburgh 40.4406° N, 79.9959° W	Began in January 2000	At ≤20 wk	**2nd trimester**	69.9 (59.0-82.9)*	ELISA	≥30 kg/m^{2}**	**Obese**
39	Gerrit van den Berg, 2013	Cohort study	372	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	49.8 ± 23.7	ELISA	>25 kg/m^{2} **	**Overweight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	58.3 ± 24.1	ELISA	>25 kg/m^{2} **	**Overweight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	62.6 ± 27.5	ELISA	>25 kg/m^{2}**	**Overweight**

39	Gerrit van den Berg, 2013	Cohort study	1902	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	63.6 ± 39.8	ELISA	<25 kg/m^2 **	**Normal weight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	71.5 ± 28.4	ELISA	<25 kg/m^2 **	**Normal weight**
39	Gerrit van den Berg, 2013	Cohort study	N/A	Amsterdam, Netherlands 52.3702° N, 4.8952° E	January 2003 to March 2004	~12 wk	**1st trimester**	69.1 ± 27.0	ELISA	<25 kg/m^2**	**Normal weight**
43	Bartoszewicz, 2013	Cohort study	13/39	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 – – March 31) and summer (April 1 – September 30)	1st trimester	**1st trimester**	68.2 ± 19	ECLIA	low BMI	**Normal weight**
43	Bartoszewicz, 2013	Cohort study	14/42	Warsaw, Poland 52.2297° N, 21.0122° E	Winter (October 1 – – March 31) and summer (April 1 – September 30)	1st trimester	**1st trimester**	58.7 ± 26	ECLIA	High BMI	**Overweight & Obese**
46	Karlsson, 2014	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	11.9 ± 1.0 wk	**1st trimester**	64.2 ± 18.3	CLIA	22.0 ± 1.4	**Normal weight**
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	11.5 ± 1.5 wk	**1st trimester**	49.7 ± 11.5	CLIA	33.9 ± 3.3	**Obese**

46	Karlsson, 2014	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	11.9 ± 1.0 wk	**2nd trimester**	58.2 ± 18.3	CLIA	22.0 ±1.4#	**Normal weight**	
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	11.5 ± 1.5 wk	**2nd trimester**	49.7 ± 18.9	CLIA	33.9 ± 3.3#	**Obese**	
46	Karlsson, 2014	Prospective study	80	Western Sweden region 57° N	April 2009 to November 2012	3rd trimester	**3rd trimester**	51.7 ± 18.3	CLIA	22.0 ± 1.4#	**Normal weight**	
46	Karlsson, 2014	Prospective study	25	Western Sweden region 57° N	April 2009 to November 2012	3rd trimester	**3rd trimester**	47.7 ± 18.3	CLIA	33.9 ± 3.3#	**Obese**	
47	Riaz, 2018	Prospective study	301	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.78 ± 2.30	**1st trimester**	40.9 ± 32.3	ELISA	22.4 ± 4.6	**Normal weight**	All women
47	Riaz, 2018	Prospective study	63	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.54 ± 2.40	**1st trimester**	43.7 ± 30.7	ELISA	17.2 ± 1.0	**Underweight**	
47	Riaz, 2018	Prospective study	130	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.79 ± 2.25	**1st trimester**	38.3 ± 29.4	ELISA	20.9 ± 1.3	**Normal weight**	
47	Riaz, 2018	Prospective study	108	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	9.92 ± 2.30	**1st trimester**	42.4 ± 36.3	ELISA	27.3 ± 4.0	**Overweight**	
47	Riaz, 2018	Prospective study	121	Karachi, Pakistan 24.8607° N, 67.0011° E	January 2012 to May 2014	Cord	**Cord**	47.3 ± 35.8	ELISA	22.4 ± 4.6	**Normal weight**	

48	Woolcott, 2016	CCS	1002	Quebec 52° 56' N / 73° 32' W and Halifax 44° 38' N , 63° 34' W , Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	54.6 ± 16.8	CLIA	<25 kg/m^2**	**Normal weight**
48	Woolcott, 2016	CCS	311	Quebec 52° 56' N / 73° 32' W and Halifax 44° 38' N , 63° 34' W , Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	51.0 ± 15.6	CLIA	25-<30 kg/m^2**	**Overweight**
48	Woolcott, 2016	CCS	124	Quebec 52° 56' N / 73° 32' W and Halifax 44° 38' N , 63° 34' W , Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	48.2 ± 16.5	CLIA	30-<35 kg/m^2**	**Obese**
48	Woolcott, 2016	CCS	84	Quebec 52° 56' N / 73° 32' W and Halifax 44° 38' N , 63° 34' W , Canada	2002–2010	12-15 wk	**1st & 2nd trimester**	45.7 ± 15.4	CLIA	≥35 kg/m^2**	**Obese class 2**
49	Valkama, 2018	Prospective study	43	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	74 ± 23	N/A	<25 kg/m^2	**Normal weight**
49	Valkama, 2018	Prospective study	34	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	73 ± 32	N/A	25-<30 kg/m^2	**Overweight**
49	Valkama, 2018	Prospective study	84	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	64 ± 24	N/A	30-<35 kg/m^2	**Obese**

49	Valkama, 2018	Prospective study	58	Helsinki, Finland 60.1699° N, 24.9384° E	2008-2014	7-18 wk	**1st & 2nd trimester**	57 ± 22	N/A	≥35 kg/m^2	**Obese class 2**

BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO).
CS- cross section, CCS-case control study, N/A not available, wk- week gestational.
** Pre-pregnancy BMI
# BMI from first-trimester

Chapter 3:

The association of overweight and obesity on vitamin D status during pregnancy using data from the FASSTT Study

Abstract

Maternal obesity and vitamin D deficiency in pregnancy are both global public health issues and obesity is a recognised risk factor for low vitamin D status. Sufficient maternal vitamin D status (25 hydroxyvitamin D (25(OH)D) concentration) is essential for optimal fetal bone growth and development. The aim of this study was to investigate the association between BMI and maternal vitamin D status. Data and samples from a previous study (FASSTT trial) were used for analysis. Pregnant women without pregnancy complications, aged 18 to 35 years, having a singleton pregnancy and not taking vitamin D supplements were included. Anthropometric measures were recorded at approximately 14 weeks gestation. Non-fasting blood samples were collected at 14 and 36 weeks gestation and analysed for total serum 25(OH)D, using liquid chromatography tandem mass spectrometry. Data from 216 pregnant women (135 normal weight, 57 overweight, 24 obese) were available for analysis. Pregnant women with obesity had significantly lower median (IQR) vitamin D status when compared to women of normal weight at 14 weeks (32.1 (18.7, 61.3) *vs* 46.1 (30.3, 63.4) nmol/L; *P*=0.038) but not at 36 weeks gestation (34.6 (19.7, 55.6) *vs* 42.1 (27.5, 65.9) nmol/L; *P*= 0.370) respectively. At both 14 and 36 weeks gestation, 60% of participants were classified as vitamin D deficient (25(OH)D <25nmol/L) or insufficient (25(OH)D 25-50nmol/L); with 37.5% and 41.7% of pregnant women with obesity classified as deficient at 14 weeks and 36 weeks gestation compared to 15.6% and 17.5% of normal weight women respectively. When examined in relation to seasonality, pregnant women with obesity had significantly lower vitamin D status during winter months when compared with women who were normal weight or overweight. Without vitamin D supplementation, there was a high prevalence of vitamin D deficiency/insufficiency in pregnant women in Northern Ireland. Maternal

obesity influences on vitamin D status, particularly in early pregnancy and during the winter months.

Introduction

Maternal obesity (BMI >30kg/m^2) is becoming increasingly prevalent and significantly contributes to adverse pregnancy and birth outcomes (Chu *et al.,* 2009; Heslehurst *et al.,* 2011; Yu *et al.,* 2013). Currently in the UK, 20% of women are classified as obese at their first antenatal appointment (Maternity Services, 2017). Obesity during pregnancy increases the risk of metabolic dysregulation, development of gestational diabetes mellitus (GDM) (Martin *et al.,* 2015; Athukorala *et al.,* 2010), pre-eclampsia (Sohlberg *et al.,* 2012) and large-for-gestational age neonates (Wang *et al.,* 2015). These conditions may be related to a decrease in insulin sensitivity in pregnant women with obesity (Catalano *et al.,* 2010). Furthermore, maternal obesity has been associated with a higher prevalence of post-term delivery (Halloran *et al.,* 2012) and an increased need for induction of labour (Wolfe *et al.,* 2011) contributing to longer duration and poorer progress in labour (Norman *et al.,* 2012) and further exacerbating the likelihood of assisted delivery. The rate of induction failure increases progressively with increasing BMI (Wolfe *et al.,* 2011) and pregnant women with obesity are 36.8% more likely to deliver via caesarean section (CS) (Dzakpasu *et al.,* 2014). Low maternal vitamin D status (measured as 25-hydroxyvitamin D (25(OH)D) concentrations) is also a risk factor for pre-eclampsia (Robinson *et al.,* 2013) and GDM (Wagner *et al.,* 2012), as vitamin D plays an important role in glucose homeostasis and insulin resistance (Sung *et al.,* 2012). Studies have also reported an association between low maternal vitamin D status, preterm delivery and hypertension, both factors also associated with higher BMI (Wagner *et al.,* 2012; Dawodu *et al.,* 2011). Furthermore, maternal vitamin D deficiency (25(OH)D < 25 nmol/L) has been significantly correlated with low neonatal birth weight and impaired

growth and bone development in the fetus (Khalessi *et al.,* 2015; Robinson *et al.,* 2011; Aghajafari *et al.,* 2013) and throughout life (Javaid *et al.,* 2006).

Vitamin D is synthesised in the skin following sunlight exposure, and with the UK's geographical latitude this synthesis is non-existent for 6 months of the year (SACN, 2016). Food sources of vitamin D are limited and include fatty fish, egg yolks, liver, mushrooms and fish-liver oils (Holick, 2007&2008) and dietary intakes of these foods can be further reduced during pregnancy (Food Standards Agency, 2008). In the UK, the mean daily intake of vitamin D from food sources for women aged 19-64 years ranged between 2.2 and 2.8μg/d (National Diet and Nutrition Survey NDNS, 2008/09 to 2011/12, Bates, 2014), well below the currently recommended intake of 10μg/d for all adults including pregnant women (SACN, 2016).

Obesity has been shown to be a risk factor for low vitamin D status (Vimaleswaran *et al.,* 2013; Earthman *et al.,* 2012) with proposed mechanisms including sequestration of this fat-soluble vitamin within adipose tissue (Holick *et al.,* 2011; Hossein-nezhad *et al.,* 2013), volumetric dilution or an overall reduction in vitamin D synthesis through reduced sun exposure (Drincic *et al.,* 2012; Pourshahidi 2015). Furthermore, it has been shown from the previous chapter (systematic review) that maternal obesity negatively influences both maternal and fetal vitamin D status. Pregnant women with obesity have significantly lower 25(OH)D concentrations compared to non-obese women (McAree *et al.,* 2013) and a twofold increase in vitamin D deficiency has been observed in pregnant women and their infants as maternal BMI increased from 22 to 34 kg/m^2 (Bodnar *et al.,* 2007).

Sufficient maternal vitamin D status is essential; the fetus is solely dependent on the mother's vitamin D supply during pregnancy (Dror *et al.,* 2010). It has been observed

that vitamin D concentrations in cord blood samples of infants born to mothers with obesity were significantly lower than those born to mothers of normal weight (Bodnar *et al.,* 2007). Furthermore, it has been suggested that pregnant women who are of a normal weight transfer more vitamin D across the placenta than pregnant women with obesity, even when maternal concentrations of 25(OH)D are equivalent (Josefson *et al.,* 2013). The association between increasing BMI and vitamin D status in UK pregnant women needs to be defined. Therefore, the primary aim of this study was to investigate the association between BMI and maternal vitamin D status. A secondary aim was to investigate for associations between vitamin D status and neonatal birth outcomes.

Methods

Participants

Data and samples from a previous trial (Folic Acid Supplementation in the Second and Third Trimesters (FASSTT)), which took place from September 2005 to December 2006 and carried out within the Nutrition Innovation Centre for Food and Health (NICHE) at Ulster University, were used for the current analysis. The methodology of the original FASSTT study is described in detail elsewhere (McNulty *et al.,* 2013). In brief, pregnant women aged 18 to 35 years, in the first trimester of pregnancy, without pregnancy complications and having a singleton pregnancy, who attended antenatal clinics at the Causeway Hospital, Coleraine United Kingdom, were recruited to the study and followed up until delivery. For the duration of the study, participants were asked to refrain from taking dietary supplements.

Ethical approval for the original study was granted by the Office for Research Ethics Committees Northern Ireland (05/Q2008/21) and written informed consent was obtained from each participant. For the current analysis, ethical approval was granted by the National Research Ethics Committee (14/0002).

Recruitment and initial sampling commenced in the first trimester at approximately 14 weeks gestation. Weight (kg) and height (cm) were measured at 14 weeks gestation by the researcher at the antenatal clinic and Body Mass Index (BMI; kg/m^2) was calculated and categorised according to WHO classifications as normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) or obese ($\geq$30.0 kg/m^2). In addition, information on age, parity, socio demographics, gestation duration, smoking habits and alcohol consumption were recorded. Following delivery, mode of delivery and infant characteristics including birth weight (g) (classified as low birth weight (<2500

g) or macrosomia (>4000 g)) (WHO, 2004), length (cm) and head circumference (cm) were recorded. Apgar scores were collected from maternal notes and paediatric charts; Apgar score is a test generally done at one and five minutes after birth to assess the health of new born children with scores between 7 and 10 classified as reassuring, 4 to 6 as moderately abnormal, and ≤3 regarded as critically low and requiring additional medical care (ACOG, 2014).

Blood sample analysis

Non-fasting blood samples were collected at 14 and 36 weeks' gestation by venepuncture into an evacuated tube by a trained phlebotomist. All samples were coded anonymously with each participant being allocated a unique identification number. Collected samples were kept chilled and processed (centrifugation at 3000 rpm for 15 minutes) within 3 hours of collection and serum aliquots were stored at -80°C until batch analysis.

Vitamin D status was measured in serum samples from both 14 and 36 weeks time points by quantifying and summing 25-hydroxyvitamin D_2 ($25(OH)D_2$) and 25-hydroxyvitamin D_3 ($25(OH)D_3$) to give total 25-hydroxyvitamin D (25(OH)D) concentrations using a fully validated method (Chromsystems Instruments and Chemicals GmbH, Gräfelfing, Germany; MassChrom 25-OH-Vitamin D_3/D_2) and liquid chromatography-tandem mass spectrometry (API 4000; AB SCIEX, Washington, DC, USA). Samples were batch analysed in the Biochemistry Department of St James's Hospital Dublin, Ireland, a laboratory which participates in the Vitamin D External Quality Assessment Scheme (DEQAS). Vitamin D status was classified into categories of sufficiency according to SACN guidelines and defined as

either deficient (25(OH)D ≤25nmol/L); insufficient (25-50nmol/L) or sufficient (25(OH)D ≥50nmol/L) (SACN, 2016).

Dietary intake

During the second trimester of pregnancy, participants completed a four-day estimated food diary to assess dietary intake. Mean daily nutrient intakes were analysed using WISP (version 3.0; Tinuviel Software).

Data analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences software, Version 22; IBM). Normality tests (Kolmogorov-Smirnov) were conducted and non-normally distributed data were log transformed as appropriate to approximate normality. A one-way ANOVA test was used to assess differences in maternal 25(OH)D concentrations (at 14 weeks and 36 weeks gestation) between the normal weight, overweight and obese categories. Paired samples T-tests were conducted to assess differences in 25(OH)D concentrations between 14 weeks and 36 weeks gestation within each of the BMI categories. Samples were collected between September 2005 and December 2006 and categorised by season; winter samples were defined as those collected: December-February, Spring samples as: March-May, Summer samples as: June-August and Autumn samples as: September- November. Chi-Square tests of association were used to assess associations between vitamin D status categories and other categorical variables including season of sampling, smoking and alcohol consumption. Bivariate correlations were carried out between 25(OH)D concentrations (at 14 weeks and 36 weeks gestation) and age, BMI, parity,

smoking and gestational age at delivery. In addition, bivariate correlation analysis was carried out between vitamin D status (at 14 weeks and 36 weeks gestation) and infant characteristics (weight, length and head circumference, Apgar score), controlling for BMI, age, parity, season and smoking during pregnancy. Multivariate-adjusted multiple regression models were used to assess determinants of vitamin D status at each time-point (14 weeks and 36 weeks gestation) and included BMI, age, parity, season, smoking during pregnancy and vitamin D intake. Data are presented as median (5^{th}, 95^{th} Percentile) and a *P value* <0.05 was considered significant in all analyses.

Results

Maternal characteristics

Samples and data from 216 pregnant women were available for this analysis, the median age of participants at enrolment was 28 years. At enrolment, median BMI was 23.9 (19.5, 32.5) kg/m^2 with 62.5% of women classified as normal weight, 26.4% overweight and 11.1% obese. There was no significant difference in maternal age, parity, gestational age at delivery or dietary vitamin D intakes across BMI categories (Table 1).

Infant characteristics

Data from 208 infants were available for the current analysis. There were no observed differences in infant birth weight, length or head circumference across maternal BMI categories. Normal weight mothers were significantly more likely to deliver a baby of normal weight compared to mothers who were overweight or obese (Table 1). Apgar scores at one minute did not significantly differ according to maternal BMI category; however, five minute Apgar scores were significantly lower in infants born to mothers with obesity compared to those born to normal weight mothers (9 (5, 9) vs. 9 (9, 9), P= 0.043) (Table 1).

Vitamin D status

A total of 216 and 122 maternal samples were available for measurement of vitamin D status at 14 and 36 weeks gestation respectively. In the total group, median (5th, 95th percentile) 25(OH)D concentration at 14 weeks was 43.8 (17.5, 102. 6) nmol/L and at

36 weeks was 37.8 (15.3, 109.5) nmol/L, concentrations considered as insufficient (<50 nmol/L). At 14 weeks gestation, 18%, 42.6% and 39.4% of pregnant women were classified as deficient, insufficient and sufficient respectively and 23.8%, 37.7% and 38.5% were classified as deficient, insufficient and sufficient respectively at 36 weeks gestation.

Vitamin D status was significantly lower in pregnant women with obesity compared those of normal weight at 14 weeks gestation but not at 36 weeks gestation (Table 2). Within BMI categories, there was no significant difference in vitamin D status as pregnancy progressed from 14 to 36 weeks gestation (Table 2). Figures 2a and 2b show the percentage of pregnant women categorised as vitamin D deficient, insufficient and sufficient within each BMI category. More women with obesity were defined as deficient at 14 weeks and 36 weeks compared to women of a normal weight, although values were only significantly different at 14 weeks P=0.025. Differences in vitamin D status at 14 weeks gestation between the three BMI categories were observed in winter but not at any other season: pregnant women with obesity had significantly lower vitamin D status (19.3 (16.7, 32.2) nmol/L) when compared with those of a normal weight (36.7 (27.6, 57.1) nmol/L) or overweight (41.6 (27.9, 57.0) nmol/L), P=0.013). There were no significant differences in vitamin D status between BMI categories during any season at 36 weeks gestation.

At 14 weeks gestation, season was a significant predictor of vitamin D status in both unadjusted and adjusted regression models. At 36 weeks gestation, season remained the only significant predictor of vitamin D status in adjusted models (Table 3).

Discussion

This study shows a high prevalence (60%) of vitamin D insufficiency (<50 nmol/L) among all pregnant women living in Northern Ireland, irrespective of their BMI at 14 weeks and 36 weeks gestation. Vitamin D deficiency (<25 nmol/L) at 14 and 36 weeks gestation was 18% and 23.8% respectively and insufficiency (25-50 nmol/L) was found in 42.6% and 37.7% respectively. These results are similar to a previous study of healthy Caucasian pregnant women in Northern Ireland (Holmes *et al.,* 2009) where it was reported that vitamin D insufficiency was prevalent during pregnancy. Other studies at similar latitudes confirm that low vitamin D status is a problem in pregnant women with up to 50% of women being classified as insufficient in the first trimester and deficiency ranging from 11- 28%, with higher levels of deficiency observed in wintertime (Haggarty *et al.,* 2013; Kiely *et al.,* 2016). Differences in vitamin D status between studies can depend on several factors such as season of blood sample, dietary intake, supplement use and the method used for the measurement of vitamin D (Wahl *et al.,* 2012; Hilger *et al.,* 2014). In addition, there is debate over the appropriate cut-offs used to categorise vitamin D status and this could account for variations in categorising deficiency and insufficiency. In the current analysis the most recent SACN cut-offs for defining deficiency/insufficiency have been used, whereas in USA The Institute of Medicine uses slightly higher cut-offs of <30 nmol/L for deficiency.

Research has shown that maternal obesity and low vitamin D status is frequently associated with having negative consequences for both the mother and the child (Bodnar *et al.,* 2007; Bener *et al.,* 2013). Obesity is also known to negatively impact on vitamin D status and of interest in this study, we found that while pregnant women with obesity had significantly lower vitamin D status than those of a normal weight in the first trimester particularly in the winter months, this was not observed in the third

trimester. Similar findings have been reported by Karlsson *et al.* (2015). However, other studies have shown that pregnant women with obesity are more likely to be vitamin D insufficient than those of a normal weight across all trimesters (Bodnar *et al.,* 2007; Andersen *et al.,* 2013). In the general population, obesity is thought to influence vitamin D status through sequestration in fat tissue. In pregnancy, this may be further exacerbated by physiological changes such as weight gain and haemodilution (Drincic *et al.,* 2012) which in turn may influence vitamin D status. A lower weight gain during pregnancy has been observed in women with obesity compared to those of normal weight (Sunsaneevithayakul *et al.,* 2014). Lower vitamin D status in obese individuals could be related to volumetric dilution (Drincic *et al.,* 2012). However, haemodilution and weight gain during pregnancy affect vitamin D status for both normal weight and obese pregnant women (Karlsson *et al.,* 2015), with the precise mechanism still poorly understood. Adequate vitamin D status during pregnancy is necessary through increased fetal demand for calcium (approximately 25–30 g) and vitamin D status of the fetus is directly correlated with maternal status (Dror *et al.,* 2011; El Rifai *et al.,* 2014; Godang *et al.,* 2014). Pregnant women with obesity have been shown to have a reduced placental transfer of 25(OH)D compared to women of a normal weight with the same prenatal vitamin D status (Josefon *et al.,* 2013), which may indicate a less efficient transfer of vitamin D across the placenta in maternal obesity. This finding supports the contention that obesity reduces the bioavailability of vitamin D (Wortsman *et al.,* 2000). Unfortunately, cord blood samples were not available for vitamin D analysis in the current study.

Vitamin D metabolism changes during pregnancy to meet the physiological demands of the developing fetus. The concentrations of the active hormonal form $1,25(OH)_2D_3$ in pregnant women at 12 weeks gestation have been reported to be triple those of non-

pregnant women (Hollis *et al.,* 2011), probably via increases in intestinal calcium absorption and immune adaptation to the presence of the fetus (Karras *et al.,* 2017). In order to produce and increase 1,25 $(OH)_2D_3$, there must be enough substrate vitamin D available (Hollis *et al.,* 2013). It has been reported previously that in maternal obesity, women are at risk of lower substrate of vitamin D compared to normal weight women (Bodnar *et al.,* 2007; Andersen *et al.,* 2013); in the current analysis we observed that pregnant women with obesity had a lower vitamin D status than those of normal weight, particularly in winter months. Therefore, those with maternal obesity are at risk of vitamin D deficiency and insufficiency particularly in winter months and adequate vitamin D status during pregnancy is essential to prevent risks associated with vitamin D deficiency and insufficiency.

Dietary intake from food alone is not adequate to prevent vitamin D deficiency, particularly during the winter months when the sun exposure is limited; in this study dietary intake of vitamin D was 1.92μg/d, which is lower than reported in recent NDNS survey for women, and also was lower than in European pregnant women (Karlsson *et al.,* 2014). Compounding these low intakes, pregnant women are advised to reduce intake of some of the naturally containing vitamin D rich foods such as liver and fish owing to safety issues and risk of bacterial exposure (The National Health Service, 2012; Food Standards Agency, 2008).

Obesity prior to or during pregnancy has been shown to contribute to increased risk of adverse birth outcomes such as large-for-gestational age infants (Wang *et al.,* 2015; Schummers *et al.,* 2015). In the current study, mothers who were overweight and obese were less likely to deliver babies of normal weight (> 2500g < 4000g) compared to mothers who were normal weight. Furthermore, infants born to mothers with obesity performed less well on the Apgar score at 5 minutes, indicating a negative impact of

obesity during pregnancy on infant health. Zhu *et al.*, 2015, also reported that maternal obesity was associated with lower Apgar score at 5 minutes.

This study has several strengths in that vitamin D status was assessed by measuring 25(OH)D concentrations using the gold-standard method of LC-MS/MS (Institute of Medicine, 2010) at two time points during pregnancy. Furthermore, blood sampling occurred across all seasons and data were available on dietary intake of vitamin D. Pregnant women in this study did not consume dietary supplements containing vitamin D removing this as a confounding factor and making this one of the only studies where the true association between maternal obesity and vitamin D status can be studied. It must be acknowledged that only one measure of pregnancy weight was obtained, therefore, we were unable to examine the influence of gestational weight gain on maternal vitamin D status. Furthermore, body composition changes may be more useful as it has also been shown that fat mass may be a better predictor of vitamin D status than BMI (Salehpour *et al.*, 2012). In the FASSTT trial there was a high dropout rate from 14-36 weeks, although our findings did not differ when analysis was conducted with and without dropouts.

Conclusion

In early pregnancy, women with obesity had significantly lower vitamin D status than those who were normal weight, this was evident particularly during the winter months. There was a high prevalence of vitamin D insufficiency at 14 and 36 weeks gestation among pregnant women living in Northern Ireland who were not taking a vitamin D supplement. These findings are particularly relevant in the context of the current maternal obesity epidemic and for those who do not adhere to the government recommendations on vitamin D supplementation during pregnancy.

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Table 1. Maternal and infant characteristics of all participants in the FASSTT study

	All	Normal weight	Overweight	Obesity	*P*
Maternal Characteristics	**n=216**	**n=135**	**n=57**	**n=24**	
Age (yrs)	28 (19, 34)	27 (19, 34)	29 (21, 34)	29 (19, 33)	0.126
Weight (kg)	64 (51, 89)	60 (50, 70)[a]	73 (62, 89)[b]	85 (64, 128)[c]	<0.0001
Height (cm)	1.64 (1.52, 1.75)	1.65 (1.54, 1.75)	1.65 (1.52, 1.78)	1.60 (1.27, 1.73)	0.003
BMI (Kg/m²)	23.87 (19.47,32.47)	22.53 (19.12,24.74)[a]	26.81 (25.14,29.42)[b]	32.07 (30.05,50.18)[c]	<0.0001
Parity	1 (0, 3)	1 (0, 3)	1 (0, 3)	1 (0, 3)	0.964
Gestation at Booking Visit(wk)	13 (10, 18)	13 (10, 18)	13 (11, 17)	13 (10, 18)	0.759
Gestation at Second blood sample(wk)	36 (34, 37)	36 (34, 37)	36 (34, 37)	36 (29, 37)	0.601
Gestation at Labour (delivery) (wk)	40 (37, 42)	40 (38, 42)	40 (35, 42)	40 (31, 41)	0.612
Smoking n (%)					
Smoker	48 (22.2%)	29 (21.5%)	14 (24.6%)	5 (20.8%)	<0.0001
Non smoker	168 (77.8%)	106 (78.5%)	43 (75.4%)	19 (79.2%)	<0.0001
Alcohol consumption n (%)					
Yes	8 (3.7%)	5(3.7%)	1 (1.8%)	2 (8.3%)	0.197
No	208 (96.3%)	130 (96.3%)	56 (98.2%)	22 (91.7%)	0.999
Dietary intake of vitamin D(µg/d)	1.92 (1.28, 3.19)	2.00 (1.27, 3.44)	1.76 (1.38, 2.92)	1.53 (1.05, 2.76)	0.775
Infant Characteristics	**N=208**	**N=128**	**N=57**	**N=23**	
Birth weight(gm)	3520 (2595, 4278)	3505(2598, 4109)	3650 (2559, 4376)	3480 (1398, 4335)	0.457
Low birth weight n(%)	6 (2.8%)	3 (2.3%)	2(3.5%)	1 (4.2%)	0.607
Normal weight n(%)	184 (86.8%)	116 (88.5%)	48(84.2%)	20 (83.3%)	<0.0001
Macrosomia n(%)	22(10.4%)	12 (9.2%)	7 (12.3%)	3 (12.5%)	0.062
Birth length(cm)	51 (47, 55.5)	51 (47, 55.5)	51 (46.9, 56)	51 (37.2, 55.5)	0.798
Head circumference (cm)	34.5 (32, 37)	34.5 (32, 37)	35 (31.9, 36)	34 (31, 38)	0.947
*Apgar					
1 minute	9 (7, 9)	9 (7, 9)	9 (5, 9)	9 (4, 9)	0.149
5 minutes	9 (9, 9)	9 (9, 9)[a]	9 (8, 9)	9 (5, 9)[b]	0.043

Maternal characteristics recorded at enrolment.
Data are presented as Median (5^{th}, 95^{th} Percentile)
BMI – body mass index according to WHO categorised as normal weight 18.50-24.99 / overweight ≥25.00 / obese ≥30.00 kg/m^2.
Apgar score is a test generally done at one and five minutes after birth to assess the health of new born children immediately after birth, Scores 7 and above are generally normal, 4 to 6 fairly low, and 3 and below are generally regarded as critically low.
Birth weight defined by WHO - Low birth weight<2500 g and macrosomia> 4000g.
Differences between the BMI categories were assessed by one-way ANOVA or Chi-squared test as appropriate. $P< 0.05$ considered significant. Columns with different superscript letters are significantly different from each other

Table 2. Differences in maternal vitamin D status according to BMI category

25(OH)D concentration nmol/L	N	Normal weight n(135)	Overweight n(57)	Obesity n(24)	*P*
		Median (IQR)			
14 week gestation	216	46.1 (30.3, 63.4)[a]	43 (28.6, 63.8)	32.1 (18.7, 61.3)[b]	0.038
		n(80)	**n(30)**	**n(12)**	
36 week gestation	122	42.1 (27.5, 65.9)	36.0 (20.1, 67.6)	34.6 (19.7, 55.6)	0.370
P*		**0.143**	**0.483**	**0.290**	

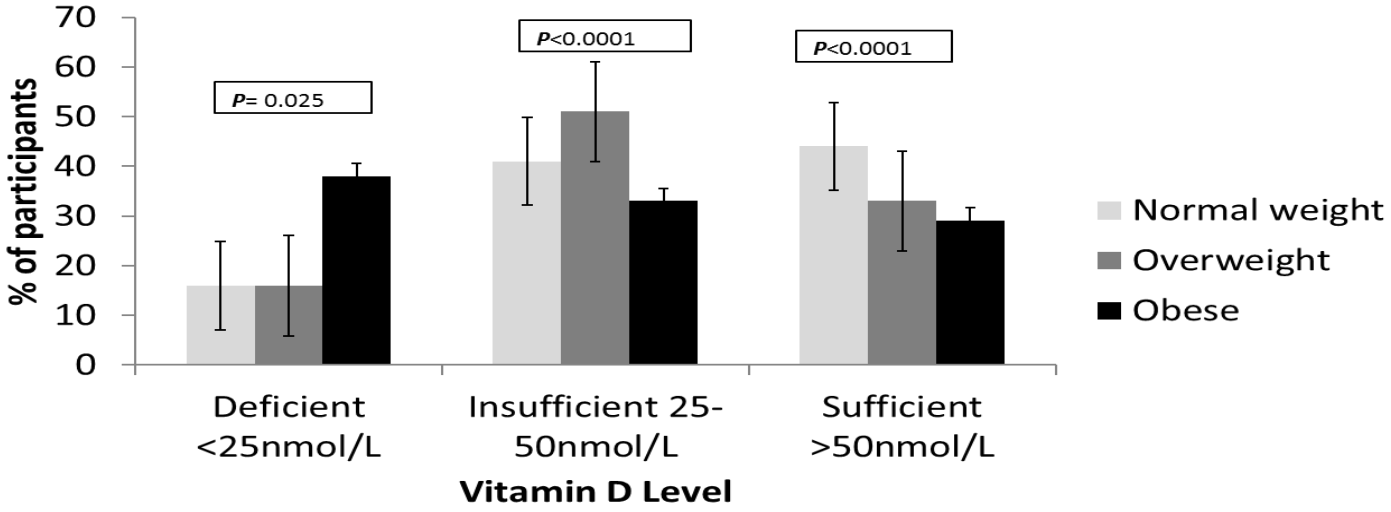
P-value obtained from one-way ANOVA test assess differences in 25(OH)D concentration between BMI categories at each time-point
*P**-value obtained from paired sample test to assess difference in 25(OH)D concentration between 14 and 36 weeks gestation within each BMI category
Data are median (5^{th}, 95^{th} percentile)

Table 3: Multiple linear regression to identify predictors of maternal 25(OH)D concentrations at 14 and 36 weeks gestation

Model	**Unadjusted 25(OH)D**				**Adjusted 25(OH)D**			
	14 weeks		**36 weeks**		**14 weeks**		**36 weeks**	
	Beta	***P***	***Beta***	***P***	***Beta***	***P***	***Beta***	***P***
BMI	-0.104	0.129	-0.201	0.026	-0.125	0.348	-0.116	0.345
Parity	0.092	0.302	-0.141	0.242	0.067	0.633	-0.130	0.304
Season*	0.275	<0.0001	0.448	<0.0001	0.303	0.027	0.349	0.007
Smoking during pregnancy	0.105	0 .123	0.318	<0.0001	0.134	0.354	0.163	0.212
Age	0.106	0.119	0.185	0.041	0.090	0.533	0.175	0.179
Vitamin D intake(nmol/L)	0.122	0.206	0.213	0.039	0.032	0.817	0.086	0.493

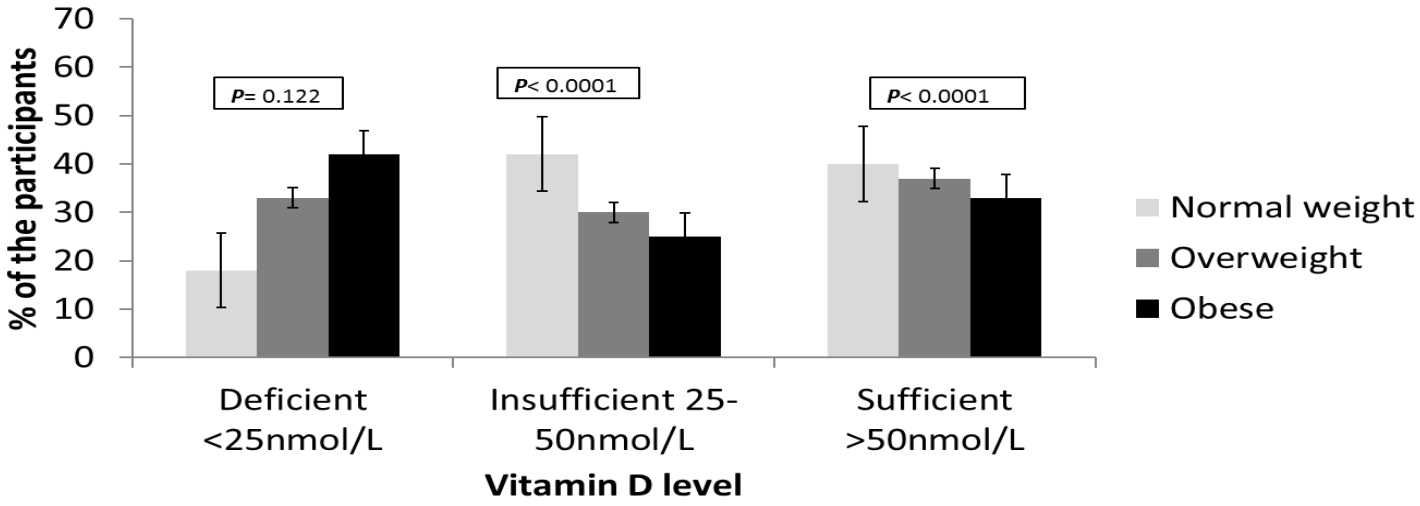
*Winter: December-February, Spring: March-May, Summer: June-August, Autumn: September- November

Figure 2a. Level of deficiency/ insufficiency and sufficiency of vitamin D at 14 weeks gestation



*Chi-square test used to assessed the differences between deficiency, insufficiency and sufficiency among BMI categories of participants at 36 weeks gestation

Figure 2b. Level of deficiency/ insufficiency and sufficiency of vitamin D at 36 weeks gestation



*Chi-square test used to assessed the differences between deficiency, insufficiency and sufficiency among BMI categories of participants at 36 weeks gestation

Chapter 4:

The association between maternal body weight and vitamin D status in early pregnancy

Abstract

Maternal BMI bas been shown to be inversely correlated with vitamin D status (25-hydroxyvitamin D (25(OH)D) concentrations) during pregnancy. Pregnant women with obesity and with vitamin D deficiency are at risk of many adverse health outcomes in pregnancy.

The aim of this study was to examine differences in maternal vitamin D status across normal weight, overweight and obese pregnant women in early pregnancy.

Data collected at baseline from a double-blind randomised vitamin D intervention study (MO-VITD) were used. Pregnant women without pregnancy complications, aged >18 years and having a singleton pregnancy were recruited between January 2016 and August 2017 at antenatal clinics in the Western Health and Social Care Trust, Northern Ireland. Non-fasting blood samples were collected at 12 weeks gestation and analysed for total serum 25(OH)D, using liquid chromatography tandem mass spectrometry. Data from 239 pregnant women (80 normal weight, 79 overweight, 80 obese) were included in the current analysis. The mean ± SD 25(OH)D concentration of all pregnant women at 12 weeks gestation was 52.0 ± 21.6 nmol/L. Pregnant women classed as obese or overweight were found to have significantly lower 25(OH)D concentrations compared to women of normal weight (48.8 ± 20.3 vs 49.8 ± 20.4 vs. 57.5 ± 23.1 nmol/L, *P*=0.019; obese, overweight, normal weight respectively). A total of 45% of all pregnant women were found to be either vitamin D deficient (25(OH)D <25nmol/L; 13%) or insufficient (25-50 nmol/L; 32%) in early pregnancy. BMI was significantly negatively correlated with 25(OH)D concentrations (***r***=-0.168; *P*=0.009). BMI (β=-0.165; *P*=0.006), season (β=0.220; *P*=<0.0001), supplement use (β=-0.268; *P*<0.0001) and a sun holiday within the previous 6 months (β=-0.180; *P*=0.010) were

significant predictors of 25(OH)D concentrations. In early pregnancy, 62% of pregnant women reported using a supplement containing vitamin D and 38% reported no supplement use. Supplement users had a significantly higher 25(OH)D concentration than non-supplement users in all BMI categories but overall, 37% of supplement users were still classified as vitamin D insufficient. Vitamin D status was significantly lower in winter months compared to summer months. In early pregnancy, especially during winter months, pregnant women with obesity, particularly non-supplement users, are at higher risk of low vitamin D status.

Introduction

The prevalence of maternal obesity is increasing, with one in five women of reproductive age in the UK being classed as obese (Poston *et el.,* 2016; Devlieger *et al.,* 2016). The UK now has the highest level of maternal obesity in Europe (Poston *et al.,* 2016), with 20% of all UK women and 17% of pregnant women in Northern Ireland classified as obese at the first antenatal appointment (Maternity Services, Monthly Statistics, 2017; Scott-Pillai *et al.,* 2013). In 2010, the European Perinatal Health Report noted that the levels of overweight or obesity in pregnant women in Poland, France and Slovenia ranged from 25.6% to 27.8%. The majority of other European countries have reported maternal overweight and obesity rates of between 30–37%, with Scotland having the highest prevalence of 48.4%, of which 20.7% were classified as obese (EURO-PERISTAT Project, 2010). Maternal obesity is associated with increased risk of maternal and perinatal mortality and morbidity, including gestational diabetes mellitus (GDM), pre-eclampsia, caesarean section (CS) and birth complications (Poston *et al.,* 2016; Sarwer *et al.,* 2006).

The adverse effects of maternal obesity are similar to those associated with vitamin D deficiency during pregnancy. Maternal vitamin D deficiency is considered a global public health concern, with approximately 23% of pregnant women observed classed as vitamin D deficient in the UK (Makgoba *et al.,* 2011) and more than 90% of pregnant women in Northern Ireland have been shown as vitamin D insufficient (Holmes *et al.,* 2009). Vitamin D (calciferol) is a fat-soluble vitamin, with a classical role in the homeostasis of calcium and phosphate for the protection and maintenance of bone health. In addition, vitamin D is involved in skeletal and non-skeletal functions (Wacker and Holick, 2013). There are two forms of vitamin D; D_3 (cholecalciferol) which is obtained from animal sources or produced from 7-dehydrocholesterol in the

skin following exposure to ultraviolet B irradiation from sunlight and D_2 (ergocalciferol) which is obtained from plant sources. Vitamin D requires hydroxylation in the liver to form 25 hydroxyvitamin D (25(OH)D) in response to circulating parathyroid hormone (PTH) concentration and a further hydroxylation in the kidneys and other organs to form the biologically active form 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) (Holick, 2007). Circulating 25(OH)D concentration is the most reliable biomarker for the measurement of vitamin D status and the Institute of Medicine (IOM) has defined serum 25(OH) D concentrations greater than 50 nmol/L as sufficient for bone health (Institute of Medicine, 2010). The cut-off point for deficiency has been defined as <25 nmol/L (SACN, 2016) or <30nmol/L (Institute of Medicine, 2011) and some researchers have argued that concentrations as high as >75 nmol/L are necessary for functions of vitamin D beyond bone health (Holick *et al.,* 2011).

Exposure to ultraviolet B-light irradiation from the sun is the main source of vitamin D, accounting for approximately 90%, while the remaining 10% comes from a small range of foods such as liver, egg yolks, fatty fish and mushrooms (Laird *et al.,* 2010). Factors such as latitude, season, sunscreen use, pollution, weather, age, skin colour and body composition are known to limit vitamin D synthesis from UVB light. During at least 6 months, over the winter period in countries at high latitudes such as the UK and Ireland, the intensity of UVB exposure from sunlight is not sufficient to support vitamin D synthesis (Institute of Medicine, 2011; Macdonald *et al.,* 2011). Obesity has been shown to influence vitamin D status, since vitamin D is fat-soluble and thus may remain hidden within the adipose tissue of overweight and obese individuals (Holick *et al.,* 2011; Holick *et al.,* 2007; Hossein-nezhad *et al.,* 2013).

Pregnant women are considered at risk of vitamin D deficiency. Hypovitaminosis D during pregnancy has been highlighted in many different geographic regions and latitudes globally (Dawodu *et al.,* 2012). In addition, obesity during pregnancy is thought to further increase the risk of both maternal and infant vitamin D deficiency as vitamin D status during pregnancy has been shown to be inversely correlated with maternal BMI (Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.,* 2013). Some studies report that obese or severely obese pregnant women have lower vitamin D status compared to non-obese women (Andersen *et al.,* 2013; Bodnar *et al.,* 2007; McAree *et al.,* 2013; Perampalam *et al.,* 2011; Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.,* 2013; Van den Berg *et al.,* 2013; Karlsson *et al.,* 2015). In a large cohort of 1345 pregnant women living in Denmark, it was reported that a 5-unit increase in BMI was associated with a 25(OH)D lowering of 4.2 nmol/L and 2.8 nmol/L in winter and summer months, respectively (Andersen *et al.,* 2013). The fetus is solely reliant on an adequate maternal supply of vitamin D (Hollis *et al.,* 1984) and neonatal vitamin D status has been shown to be highly correlated with maternal status (Dror *et al.,* 2011; El Rifai *et al.*, 2014; Godang *et al.,* 2014). Furthermore, It has been shown that neonates of obese mothers had significantly lower vitamin D status in cord blood compared to neonates of normal weight mothers (Bodnar *et al.,* 2007; Karras *et al.,* 2013) despite maternal 25(OH)D did not differ (Josefson *et al.,* 2013). Due to the high levels of both obesity and vitamin D deficiency in pregnancy, the interplay between maternal BMI and vitamin D warrants further investigation.

The aim of this study was to examine differences in maternal vitamin D status across normal weight, overweight and obese pregnant women in early pregnancy.

Methods

Participants

This study investigated pregnant women recruited to the 'Association between Maternal Body Weight and Vitamin D Status (MO-VITD) Study', which was carried out within the Nutrition Innovation Centre for Food and Health (NICHE) at Ulster University and Western Health and Social Care Trust (WHSCT) in Northern Ireland. Pregnant women (n=240, with equal numbers of normal weight, overweight and obese) were recruited between January 2016 and August 2017 during their first antenatal visit. At the booking appointment (approximately 9-10 weeks gestation), all pregnant women in the WHSCT area received an information sheet from their health care provider (HCP) with a detailed study outline and contact details of the lead researcher if any further details were required. The HCP also verbally informed the potential participant about the study. At the hospital clinic when women attended for their 12-week antenatal scan, the researcher was present to answer any further questions and to take written informed consent form eligible participants.

The inclusion criteria were: pregnant women who were at least 12 weeks gestation, aged ≥18 years, BMI ≥18.5 kg/m², without current pregnancy related complications and having a singleton pregnancy. Exclusion criteria included, aged <18 years, BMI <18.5 kg/m^2, participants with multiple pregnancy, those currently involved in another research study, participants with a history of gastrointestinal, hepatic, renal, vascular or haematological disorders. In addition, participants who have had *in vitro* fertilisation (IVF) treatment, participants with a history of NTD affected pregnancies and pregnant women with active thyroid disease (e.g., Graves, Hashimoto or thyroiditis) were also excluded.

All participants provided written informed consent according to the Declaration of Helsinki. The study was reviewed by the Biomedical Sciences Ethics Filter Committee, Ulster University (Reference:15/0041) and approved by Office for Research Ethics Committees (ORECNI) (Reference:15/NI/0068) and by Western Health and Social Care Trust (WHSCT) (Reference:WT14/49). The study was registered at ClinicalTrials.gov ID: NCT02713009.

Lifestyle and anthropometric information

All participants completed a Health and Lifestyle Questionnaire at the first antenatal visit, which recorded information on age, social demographics, medication use, supplementation use and sun exposure, including sun bed use, sunbathing habits, and whether participants had been on a sun holiday within the previous 6 months. In addition, details from maternal notes were recorded including weeks gestation, parity, blood pressure, smoking and previous miscarriage.

At the first antenatal visit, anthropometric and body composition measurements were taken, included height (using a stadiometer), weight, fat mass (kg) and fat free mass (kg)(using TANITA, MC-780MA scale). BMI (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (World Health Organisation, 2004). All measurements were carried out by trained researchers in a private environment within the clinic setting.

Blood sample analysis

Non-fasting blood samples were collected from all participants at the first antenatal visit (8-17 gestation weeks) by a fully trained phlebotomist. A total of 20 ml (2x8ml

serum tubes and 1x4ml plasma tube) of blood was collected and kept chilled until processed (centrifugation at 3000 rpm for 15 minutes) within 3 hours of collection. Plasma and serum aliquots were stored at -80°C until batch analysis. Blood samples for n=239 participants were available for analysis. One participants withdrew from the study (personal reasons) and withdrew consent for blood sample analysis. Stored serum samples were used and vitamin D analysis was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) by quantifying and summing 25-hydroxyvitamin D_2 (25(OH)D_2) and 25-hydroxyvitamin D_3 (25(OH)D_3) to give total 25-hydroxyvitamin D (25(OH)D) concentrations (Chromsystems Instruments and Chemicals GmbH, Gräfelfing, Germany; MassChrom 25-OH-Vitamin D_3/D_2 andAPI 4000 LC-MS/MS; AB SCIEX, Washington, DC, USA). Vitamin D status was classified into categories of sufficiency according to SACN guidelines and defined as deficient (25(OH)D <25nmol/L); insufficient (25-50nmol/L) or sufficient (25(OH)D>50nmol/L) (SACN, 2016). Plasma intact PTH concentrations were measured, using a commercially available enzyme-linked immunosorbent assay (MD Biosciences Inc., Minnesota, USA). Samples were batch analysed in the Biochemistry Department of St James's Hospital (Dublin, Ireland).

Serum concentrations of calcium and albumin were measured using an automated clinical chemistry system (Ilab 650 Clinical Chemistry System, WERFEN). Albumin-adjusted calcium was calculated using the following formulae:

Adjusted calcium = measured total calcium+0.02(40-albumin)

Or for albumin greater than 45g/L:

Adjusted calcium = measured total calcium-0.02(45-albumin)

Statistical analysis

The statistical analyses were performed using SPSS (Statistical Package for the Social Sciences software, Version 22; IBM). Data were assessed for normality using Kolmogorov-Smirnov test. Data were presented as mean ± SD. One-way analysis of variance (ANOVA) was used to assess differences in maternal 25(OH)D concentrations between BMI categories and independent-samples t-test was used to assess differences in maternal 25(OH)D concentrations between supplement users and non-users. Chi-Square tests were performed to determine associations between the category of vitamin D sufficiency and other categorical variables including smoking, education level, material status, parity, medication use, dietary supplement use, recent sun holiday, sunbed use and season. Bivariate correlations were performed between 25(OH)D concentrations and age, body composition measures, biochemical measures and blood pressure. In addition, multivariate-adjusted multiple regression models were used to assess determinants of vitamin D status. Results were considered significant when *P value* <0.05 in all analyses.

Results

Maternal characteristics

Samples and data from 239 pregnant women were available for this analysis. Mean ± SD age of participants at recruitment was 29.5 ± 5.2 years and length of gestation was 12.8 ± 1.4 weeks. Overall mean ± SD BMI was 27.9 ± 5.5 kg/m^2 and participants were equally distributed across the BMI categories (n=80 normal weight, n=79 overweight and n=80 obese). There was no significant difference in maternal demographic factors between the three BMI categories. As expected, body weight, BMI, and all body composition measures were significantly different across BMI categories. In addition, systolic blood pressure was significantly higher in the obese mothers compared to normal weight mothers (Table 1).

Vitamin D status

Overall the mean ± SD of 25(OH)D concentration of the cohort was 52.0 ± 21.6 nmol/L, a level considered sufficient (>50 nmol/L). In the cohort as a whole, 13.4%, 31.8% and 54.8% of pregnant women were classified as vitamin D deficient, insufficient and sufficient respectively.

25(OH)D concentrations were significantly lower in pregnant women with obesity and overweight compared to women of normal weight (48.8 ± 20.3 vs. 49.8 ± 20.4 vs. 57.5 ± 23.1 nmol/L, *P*=0.019; obese, overweight, normal-weight respectively) (Table 2). Almost two-thirds of participants reported using a vitamin D containing supplement and these supplement users had a significantly higher 25(OH)D concentration than non-supplement users in the cohort as a whole and in all BMI categories. Among supplement users, the 25(OH)D concentration was significantly lower in pregnant

women with obesity and overweight compared to women of normal weight (54.4 ± 18.3 vs. 54.4 ± 17.2 vs. 62.4 ± 16.7 nmol/L, *P*=0.019; obese, overweight, normal-weight respectively); this difference was not observed in non-supplement users (Table 2).

There was no significant difference in the percentage of participants with vitamin D deficiency, insufficiency and sufficiency across the BMI categories in the cohort as a whole or when comparing supplement users and non-supplement users (Figure 1).

Overall 25(OH)D concentrations during winter and summer months was 49.0 ± 22.4 *vs*. 55.7 ± 20.0 nmol/L respectively, with status significantly lower in winter months *(P*=0.017). The 25(OH)D concentration of overweight pregnant women was significantly lower in winter months compared to summer months 45.4 ± 20.0 vs. 54.9 ± 20.0 nmol/L, *P*= 0.038 (Figure 2). During winter months pregnant women with obesity and overweight had significantly lower 25(OH)D concentrations compared to their normal weight counterparts, however this difference was not observed in summer months.

In non-supplement users, 25(OH)D concentrations during winter and summer was significantly lower compared to supplement users. In winter months, 25(OH)D concentrations of non-supplement users with obesity was significantly lower than supplement users with obesity (32.8 ± 18.9 vs. 51.6 ± 19.4 nmol/L *P*=0.004) (Table 3).

There was a significant negative correlation between 25(OH)D concentrations and maternal fat mass (*r*= -0.212; *P*= 0.001) and a significant positive correlation between vitamin D status and maternal age (*r*= 0.181; *P*= 0.005) (Figure 3).

In early pregnancy, BMI, season, age, supplement use, sun holiday and sun bed/bathing were significant predictors of 25(OH)D concentrations in unadjusted regression models. After adjustment BMI (β=-0.165; *P*=0.006), season (β=0.220; *P*=<0.0001), supplement use (β=-0.268; *P*<0.0001) and sun holiday (β=-0.180; *P*=0.010) remained significant predictors of vitamin D status (Table 4).

Others blood biomarkers

Mean ± SD concentrations of adjusted calcium and PTH are shown in Table 5. There were no significant differences in adjusted calcium or PTH concentrations across BMI categories. A significant negative correlation was observed between 25(OH)D and PTH concentrations (r= -0.343; *P*<0.0001) (Figure 3), but no apparent correlation between 25(OH)D and adjusted calcium concentrations.

Discussion

In this study we found that pregnant women with obesity and overweight had significantly lower vitamin D status than women of normal weight in early pregnancy, irrespective of supplement use; BMI was found to be a significant predictor of vitamin D status. Other studies have reported similar findings (Perez-Lopez *et al.,* 2011; McAree *et al.,* 2013; Bartoszewicz *et al.,* 2013) and in addition, some researchers have reported this difference even when dietary intake of vitamin D was significantly higher in women with obesity compared to women of normal weight (Karlsson *et al.,* 2015). Low vitamin D status in obesity is thought to be due to sequestration in fat tissue. Physiological changes during pregnancy including weight gain, haemodilution and volumetric dilution are also postulated to influence vitamin D status (Drincic. *et al.,* 2012). However, haemodilution and weight gain during pregnancy are thought to have a similar effect on vitamin D status in pregnant women of normal weight and those with obesity (Karlsson, *et al.,* 2015), still very little is known on this issue.

This study shows that whilst the overall mean vitamin D status was considered sufficient, there was still a high prevalence (45%) of vitamin D insufficiency (<50 nmol/L) among women in early pregnancy living in Northern Ireland. These results are similar to a recent study of 144 pregnant women in Cork, Ireland (Callaghan *et al.,* 2018), which reported that whilst the mean vitamin D status was considered sufficient, 44% were vitamin D insufficient (<50 nmol/L). Other European studies have reported similar findings on a sufficient vitamin D status in pregnant women in the first trimester (Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.,* 2013). Whilst other studies conducted at similar latitudes reported an insufficient vitamin D status among pregnant women during the first trimester (Haggarty *et al.,* 2011; Savvido *et al.,* 2012; McAree *et al.,* 2013; Makgob *et al.,* 2011).

In the current study, 62% of women reported taking a vitamin D containing supplement upon entering the study, which may explain the levels of sufficiency and why supplement users had significantly higher vitamin D status than non-supplement users, similar to the findings of Holmes *et al.* (2009). Pregnant women who were not taking a vitamin D supplement in early pregnancy were classified as vitamin D insufficient. Sufficiency, insufficiency and deficiency of vitamin D in non-supplement users was 40.7%, 29.7% and 29.7% respectively compared to supplement users 63.3%, 33.3% and 3.4% respectively. Across BMI categories, there was no significant difference in the prevalence of vitamin D deficiency, insufficiency or sufficiency for either supplement or non-supplement users.

Insufficiency of vitamin D was higher among pregnant women with obesity than in those who were overweight or normal weight (51.3% vs 49.4% vs 35% respectively). The level of vitamin D insufficiency increased among pregnant women who were not taking a supplement in early pregnancy from 48.1% to 62.5% to 65.5%, in those who were considered as normal weight, overweight and obese respectively. Previous studies have similarly reported that the prevalence of vitamin D deficiency is higher among pregnant women with obesity compared to non-obese pregnant women (Perez-Lopez *et al.,* 2011; McAree *et al.,* 2013). This result shows the importance of vitamin D supplementation in early pregnancy for those women living in northern latitudes to help reach sufficient levels of vitamin D, particularly for those who are overweight or obese.

In the current study, we found seasonal differences in the vitamin D status of pregnant women, with significantly lower vitamin D status in winter months compared to summer months and a corresponding higher rate of deficiency and insufficiency during winter months. Similar findings in Northern Ireland have been reported by Holmes *et*

al. (2009). This has also been noted previously in a large prospective study of 995 pregnant women in the first trimester at a similar UK latitude (Savvido *et al.,* 2012). Vitamin D status of non-supplement users was highly influenced by season, where vitamin D was lower in non-supplement users in both winter and summer months. Vitamin D supplementation in early pregnancy is considered to be one of the factors associated with the change in season-corrected vitamin D, a negative association has been observed when comparing season-corrected 25(OH)D concentrations between women who never took supplements and those who previously had but discontinued during early pregnancy (Moon *et al.,* 2015). Vitamin D status of pregnant women with obesity and overweight was also affected by season, particularly in winter months when compared to women of normal weight. Therefore, pregnant women with obesity and overweight are at risk of low vitamin D status particularly in winter months. Adequate vitamin D status during pregnancy is essential to prevent risks associated with vitamin D deficiency and insufficiency.

BMI, season, sun holiday and supplement use were significant predictors of vitamin D status; this is similar to findings from a Scottish study in pregnant women at 19 weeks gestation, where month of blood sampling, dietary vitamin D intake and vitamin D supplement use were predictors of vitamin D status (Haggarty *et al.,* 2011). Previous research has indicated that these factors are also predictors of vitamin D status in non-pregnant populations (Tsiaras and Weinstock, 2011).

In this study maternal vitamin D status was significantly negatively correlated with PTH concentrations, similar to the findings of Callaghan *et al.* (2018). However, there was no association between maternal BMI and adjusted calcium or PTH concentrations.

This study has several strengths in that vitamin D status was assessed by quantifying 25(OH)D concentrations using the gold-standard method of LC-MS/MS (Institute of Medicine, 2010). Furthermore, BMI and vitamin D were measured at the same time allowing the accurate assessment of the association between body weight and vitamin D status, with equal numbers of pregnant women across the BMI categories. Moreover, the blood sampling for vitamin D measurement occurred across all seasons allowing for the impact of seasonal variation to be investigated. The use of vitamin D containing supplements was recorded although we had no measure of dietary vitamin D intake in early pregnancy.

Conclusion

Pregnant women with overweight and obesity had significantly lower vitamin D status compared to pregnant women of normal weight in early pregnancy and BMI was found to be a significant negative predictor of vitamin D status. There was a high prevalence of vitamin D insufficiency among pregnant women living in Northern Ireland, and this was highest among non-supplement users in winter months. These findings are important for public health agencies when considering recommendations for vitamin D supplementation both pre and during pregnancy.

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Table 1: Baseline characteristics in all pregnant women and across BMI categories in participants on the MO-VITD study.

	All	Normal weight	Overweight	Obese	*P*
	n 239	n 80	n 79	n 80	
Age (yrs)	29.5 ± 5.2	29.9 ± 5.1	29.5 ± 5.5	29.2 ± 5.1	0.529
Weight (kg)	74.5 ± 15.8	59.7 ± 6.6	72.4 ± 6.1	91.5 ± 12.5	<0.0001
Height (m)	1.63 ± 0.61	1.63 ± 0.06	1.63 ± 0.06	1.63 ± 0.05	0.947
BMI (kg/m^2)	27.9 ± 5.5	22.3 ± 1.7^a	27.1 ± 1.4^b	34.2 ± 3.8^c	<0.0001
Fat (%)	34.0 ± 6.8	27.6 ± 5.5^a	34.7 ± 3.7^b	39.6 ± 4.6^c	<0.0001
Fat mass (kg)	26.5 ± 10.5	17.7 ± 7.9^a	26.1 ± 6.2^b	35.7 ± 8.2^c	<0.0001
Fat free mass (kg)	48.0 ± 10.5	41.9 ± 7.1^a	46.2 ± 5.7^b	55.8 ± 12.2^c	<0.0001
Fat mass index (kg/m^2)	9.9 ± 3.8	6.6 ± 2.7^a	9.7 ± 2.1^b	13.3 ± 2.9^c	<0.0001
Fat free mass index (kg/m^2)	17.9 ± 3.6	15.7 ± 2.4^a	17.3 ± 1.9^b	20.8 ± 4.1^c	<0.0001
Gestational weeks (wk)	12.8 ± 1.4	12.9 ± 1.3	12.8 ± 1.5	12.8 ± 1.3	0.945
Blood pressure(mmHg)					
Systolic	119.9 ± 11.6	117.2 ± 11.9^a	120.4 ± 12.2	122.2 ± 10.2^b	0.011
Diastolic	72.1 ± 8.9	70.6 ± 9.2	73.3 ± 9.3	72.4 ± 8.1	0.115
Smoker n (%)	30 (12.7)	8 (10)	11 (13.8)	11 (14.1)	0.679
Parity n(%)					
0	96 (40.7)	28 (35.4)	35 (44.3)	33 (42.3)	0.826
1	82 (34.7)	30 (38)	25 (31.3)	27 (34.6)	
2^+	58 (24.6)	21 (26.6)	19 (24.1)	18 (23.1)	
Previous miscarriage n(%)	71 (29.7)	24 (30)	26 (32.9)	21 (26.3)	0.654
Education level n(%)					
Secondary	74 (31.8)	16 (20.5)	25 (32.1	33 (42.9)	0.033
Diploma	41 (17.6)	12 (15.4)	15 (19.2)	14 (18.2)	
Degree	76 (32.6)	32 (41)	24 (30.8)	20 (26)	
Postgraduate	30 (12.9)	16 (20.5)	8 (10.3)	6 (7.8)	
other	12 (5.2)	2 (2.6)	6 (7.7)	4 (5.2)	
Marital status n(%)					
Married	120 (50.8)	46 (57.5)	40 (50.6)	36 (45)	0.285
Unmarried	117 (49.0)	34 (42.5)	39 (49.4)	44 (55)	
Regular medication n(%)	23 (9.6)	9 (11.3)	7 (8.8)	7 (8.8)	0.833
Medical illnesses n(%)	22 (9.2)	8 (10)	5 (6.3)	9 (11.3)	0.537
Vitamin D supplement n(%)					
Supplement user	147 (61.8)	53 (66.3)	46 (59.0)	48(60)	0.55
Non-user	91 (38.2)	27 (33.8)	32 (41.0)	32 (40)	
Sun holiday past 6 mth n(%)	43(18.1)	13 (16.3)	12 (15.4)	18 (22.5)	0.512
Sun bathing/bed 1 mth n(%)	18 (7.6)	7 (8.9)	6 (7.7)	5 (6.3)	0.782

Data are presented as mean ± SD or n (%).
BMI – body mass index according to WHO categorised as normal weight 18.5-24.9 / overweight ≥25.0 / obese ≥30.0 kg/m^2.
Fat mass index (kg/m^2) Fat mass (kg) divided by height^2 (m^2), Fat free mass index (kg/m^2) Fat free mass (kg) divided by height^2 (m^2). mth indicates month.
Differences between the BMI categories were assessed by ANOVA or Chi-squared test as appropriate, (P<0.05) considered significant. Columns with different superscript letters are significantly different from each other.

Table 2: Maternal 25(OH)D concentration across BMI categories in all and within supplement users and non-supplement users

25(OH)D (nmol/L)	**All**	**Normal weight**	**Overweight**	**Obese**	***P***
All	n=239	n=80	n=79	n=80	
	52.0 ± 21.6	57.5 ± 23.1[a]	49.8 ± 20.4[b]	48.8 ± 20.3[b]	0.014
Supplement user	n= 147	n=53	n=46	n=48	
	57.3 ± 17.7	62.4 ± 16.7[a]	54.4 ± 17.2[b]	54.4 ± 18.3[b]	0.045
Non-supplement user	n=91	n=27	n=32	n=32	
	43.6 ± 24.6	47.9 ± 30.2	43.1 ± 23.2	40.3 ± 20.6	0.706
P	<0.0001	0.039	0.025	0.002	

Data are presented as mean ± SD.

Differences of maternal 25(OH)D between BMI categories were assessed by ANOVA, differences between supplement users and non-supplement users assessed by independent sample t-test. ($P<0.05$) considered significant. Columns with different superscript letters are significantly different from each other.

Table 3: Seasonal difference on maternal 25(OH)D concentration within supplement user and non-supplement user across BMI categories

25(OH)D (nmol/L)	**All**	**Normal weight**	**Overweight**	**Obese**	***P***
Winter					
Supplement user	(80) 54.4 18.5	(30) 61.1 18.2[a]	(24) 49.0 16.0[b]	(26) 51.6 19.4	0.035
Non-supplement user	(48) 39.1 24.9	(15) 43.7 30.8	(18) 40.5 24.0	(15) 32.8 18.9	0.474
P	<0.0001	0.057	0.205	0.004	
Summer					
Supplement user	(67) 60.9 16.2	(23) 64.3 14.9	(22) 60.3 16.9	(22) 57.9 16.8	0.420
Non-supplement user	(43) 48.7 23.7	(12) 53.4 30.0	(14) 46.6 22.7)	(17) 47.1 20.4	0.729
P	0.004	0.257	0.064	0.077	

Data are presented as (n) mean ± SD.

Differences of maternal 25(OH)D between BMI categories were assessed by ANOVA, differences between supplement users and non-supplement users assessed by independent sample t-test. ($P<0.05$) considered significant. Columns with different superscript letters are significantly different from each other.

Table 4: Multiple linear regression to identify predictors of maternal 25(OH)D concentrations at early pregnancy

Model	**Unadjusted 25(OH)D**		**Adjusted 25(OH)D**	
	Beta	***P***	***Beta***	***P***
BMI categories	-0.135	0.037	-0.165	0.006
Season*	0.168	0.009	0.220	<0.0001
Age	0.216	0.001	0.099	0.107
Supplement use	-0.308	<0.0001	-0.268	<0.0001
Sun holiday	-0.192	0.003	-0.180	0.010
Sunbed	-0.165	0.011	-0.057	0.400
Parity	0.016	0.810		
Education	0.022	0.743		

* Winter: October-March, Summer: April-September. ($P<0.05$) considered significant.

Table 5: Maternal blood biomarkers in early pregnancy

	All	**Normal weight**	**Overweight**	**Obese**	***P***
	n 239	**n 80**	**n 79**	**n 80**	
Adjusted calcium (mmol/L)	2.29 ± 0.11	2.28 ± 0.12	2.29 ± 0.10	2.31 ± 0.09	0.139
Calcium (mmol/L)	2.29 ± 0.11	2.29 ± 0.13	2.29 ± 0.10	2.31 ± 0.10	0.196
Parathyroid hormone (pg/ml)	21.6 ± 8.4	21.3 ± 7.5	20.7 ± 9.4	22.6 ± 8.3	0.383

Data are presented as mean ± SD.
Differences of maternal biomarkers between BMI categories were assessed by ANOVA ($P<0.05$) considered significant.
Columns with different superscript letters are significantly different from each other.

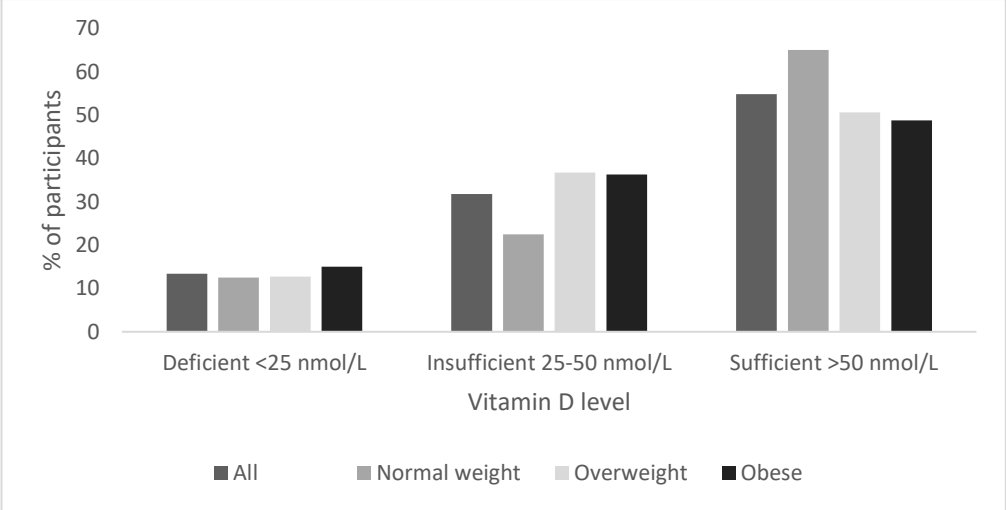

Figure 1: Level of deficiency/ insufficiency and sufficiency of vitamin D in early pregnancy

Differences between level of vitamin D deficiency, insufficiency and sufficiency among BMI categories were assessed by chi-square test, (P<0.05) considered significant.

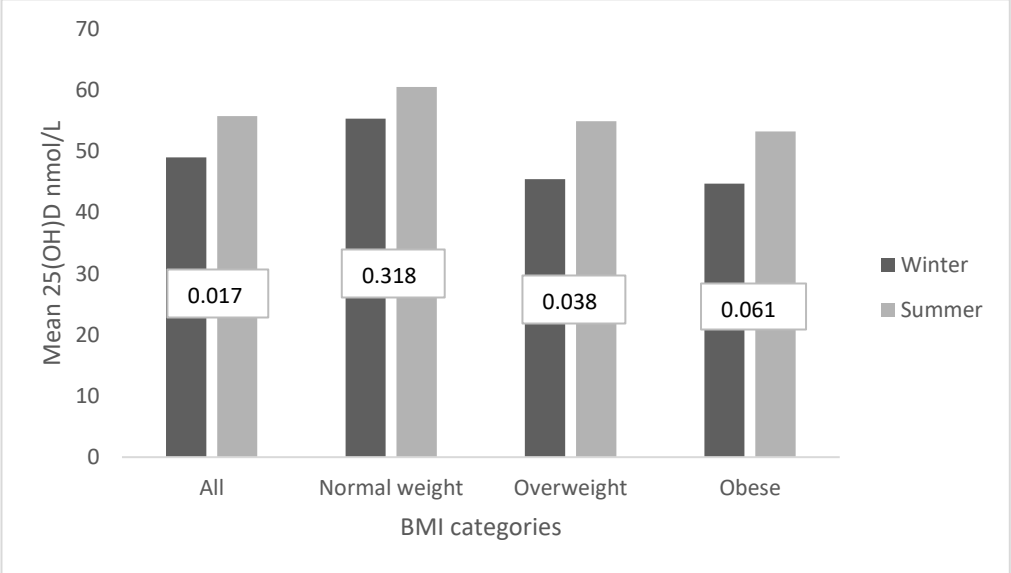

Figure 2: Seasonal differences on maternal 25(OH)D concentrations across BMI categories.

Differences in 25(OH)D concentrations between season were assessed by independent sample t-test, (P<0.05) considered significant.

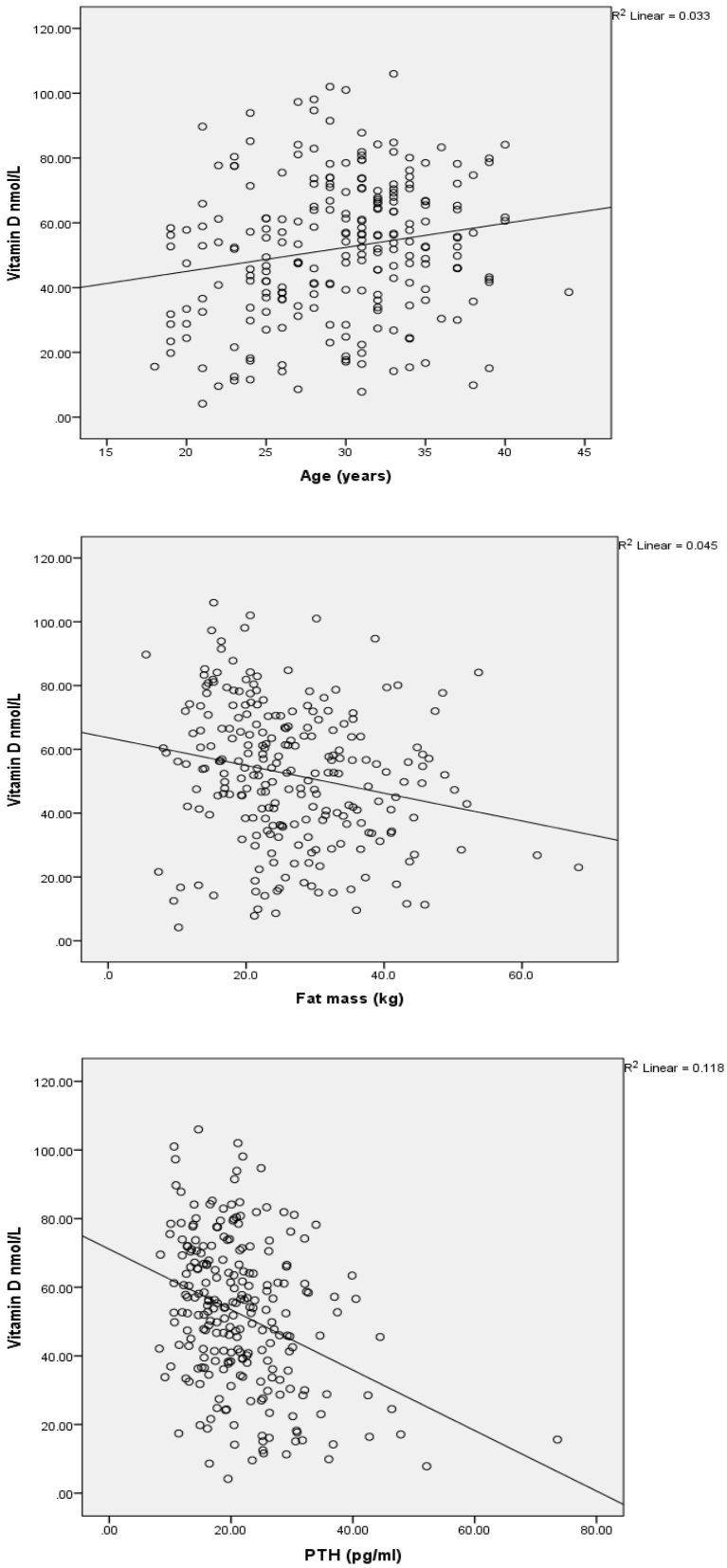

Figure 3: Correlation between 25(OH)D concentrations at early pregnancy and (age, fat mass, PTH)

Chapter 5:

The effect of supplementation of 10µg vs 20µg vitamin D_3/d on vitamin D status in normal weight, overweight and obese pregnant women

Abstract

The level of maternal and infant adequacy in vitamin D status achievable by the current recommendation of 10µg/d remains controversial. Pregnant women who are overweight or obese at the beginning of pregnancy may be particularly vulnerable to low vitamin D status owing to the higher requirement and lower vitamin D status associated with obesity

The aim of the current study was to assess the effect of supplementation of 10µg vs 20µg vitamin D_3/d throughout pregnancy on vitamin D status of normal weight, overweight and obese pregnant women and on the vitamin D status of cord blood of their infants.

Data collected from a double-blind randomised vitamin D intervention study (MO-VITD) were used. A total of 240 pregnant women were recruited throughout the year at antenatal clinics in Northern Ireland, with equal numbers in each BMI category (normal weight, overweight, obese). Pregnant women were assigned to receive 10µg or 20µg vitamin D from 12 weeks gestations until delivery. Non-fasting maternal blood samples were collected at 12, 28 and 34-36 weeks gestation and from cord after delivery and analysed for total serum 25(OH)D using liquid chromatography tandem mass spectrometry.

There was a high prevalence of vitamin D insufficiency in the 1st trimester in both the 10µg and 20µg groups (41.5 % and 48.8% respectively) with no statistical difference between treatment groups. Maternal 25(OH)D concentrations, increased from the 1st trimester to 3rd trimester in both the 10µg and 20µg groups, this increase was higher in the 20µg group (17.1 ± 24.7 and 28.8 ± 33.3 nmol/L, P=0.002). There was no overall statistical difference in cord blood 25(OH)D concentrations between the treatment

groups. In the 20µg group, maternal and cord blood 25(OH)D concentrations reached and maintained sufficiency as (≥50 nmol/L) throughout the pregnancy, even in those who started pregnancy with an insufficient status. In obese women who started pregnancy with an insufficient vitamin D status, the related cord blood 25(OH)D concentrations were deficient in both the 10µg and 20µg groups (19.4 ± 20.2 vs. 19.5 ± 9.4 nmol/L, *P*= 0.992) respectively. In contrast, obese women who started pregnancy with a sufficient status had 25(OH)D cord blood concentrations above deficiency and within the insufficiency range in both the treatment groups.

Maternal vitamin D supplementation of 20µg/d is needed to reach and maintain maternal and cord vitamin D status ≥50 nmol/L during pregnancy in pregnant women living in Northern Ireland.

Introduction

Obesity is an additional risk factor for low vitamin D status for both the mother and infant. Previous studies have found a negative association between higher body weight and vitamin D deficiency during pregnancy (Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.*, 2013). Many reasons have been proposed for this association including the fat-solubility of vitamin D and its sequestration within adipose tissue (Holick *et al.,* 2011; Hossein- nezhad *et al.,* 2013); other have suggested it may be attributed to volumetric dilution or factors such as less sun exposure (Drincic *et al.,* 2012; Pourshahidi, 2015). Furthermore, it has been shown that maternal obesity negatively influences both maternal and fetal vitamin D status with obese pregnant women having significantly lower vitamin D status compared to their non-obese counterparts (McAree *et al.*, 2013). It was observed among 1345 pregnant women living in Denmark that a 5-unit increase of BMI during winter and summer correlated to lower 25(OH)D of 4.2 nmol/L and 2.85 nmol/L respectively (Andersen *et al.,* 2013). The in-utero environment affects fetal development; maternal vitamin D supply is the main source of fetal vitamin D (Hollis *et al.,* 1984) and vitamin D status of the neonate has been shown to be highly correlated with maternal vitamin D status (Dror *et al.,* 2011; El Rifai *et al.,* 2014; Godang *et al.*, 2014) accounting for 60-80% of infants' status at delivery (Hollis *et al.,* 1984; Vieth *et al.,* 2013). Vitamin D deficiency during pregnancy is associated with poor fetal and postnatal growth (Weinert *et al.,* 2015; Leffelaar *et al.,* 2010; Robinson *et al.,* 2011). It has been observed that neonates of obese mothers had significantly lower vitamin D status in cord blood compared to neonates of normal weight mothers (Bodnar *et al.,* 2007; Karras *et al.,* 2013) despite no difference in maternal 25(OH)D status (Josefson *et al.,* 2013). These findings are

suggestive of the potential need for a higher level of vitamin D supplementation for pregnant women who are overweight or obese.

The effect of vitamin D supplementation on maternal and neonatal outcomes has been examined in previous systematic reviews (De-Regil *et al.*, 2012& 2015; Harvey *et al.*, 2014; Perez-Lopez *et al.*, 2015; Thorne-Lyman and Fawzi 2012), however a dearth of knowledge remains as to how maternal body weight affects this relationship. Previous studies have not accounted for the influence of maternal body weight on vitamin D status. In a UK study by Yu *et al.,* 2009, a single oral dose of 5000 µg/d vitamin D was compared to a daily supplement of 20µg/d vitamin D from 27 weeks until delivery and a group that received no treatment. The supplementation groups had significantly higher vitamin D status at 34 weeks compared to the no treatment group, however the authors did not account for the possible effect of maternal BMI. More recently in 2016, Cooper *et al.,* conducted a multicentre study where pregnant women were randomly assigned to either 25µg/d vitamin D_3 or matched placebo from 14 weeks gestation until delivery. Vitamin D status was significantly higher in the supplement group compared to placebo at 34 weeks gestation, however maternal BMI at baseline was higher in the placebo group compared to the supplement group. Information regarding the relationship between maternal weight and vitamin D status remains scarce, therefore the aim of the current study was to assess the effect of supplementation of 10µg vs 20µg vitamin D_3/d throughout pregnancy on vitamin D status of normal weight, overweight and obese pregnant women and on the cord blood of their infants.

Methods

Study design (Figure 1)

A double-blind randomised vitamin D intervention study (MO-VITD), conducted and reported according to consort guideline (Consort, 2010). Two-hundred and forty pregnant women received either 10μg or 20μg vitamin D from 12 weeks gestation through to delivery.

Participants

This study recruited pregnant women to the 'Association between Maternal Body Weight and Vitamin D Status (MO-VITD) Study', which was carried out within the Nutrition Innovation Centre for Food and Health (NICHE) at Ulster University and the Western Health and Social Care Trust (WHSCT) in Northern Ireland. Pregnant women (n=240, with equal numbers of normal weight, overweight and obese) were recruited between January 2016 and August 2017 during their first antenatal visit. At the booking appointment (approximately 9-10 weeks gestation), all pregnant women in the WHSCT area received an information sheet from their health care provider (HCP) with a detailed study outline and contact details of the lead researcher if any further details were required. The HCP also verbally informed the potential participant about the study. At the hospital clinic when women attended for their 12-week antenatal scan, the researcher was present to answer any further questions and to take written informed consent from eligible participants.

The inclusion criteria were: pregnant women of at least 12 weeks gestation, aged ≥18 years, BMI ≥18.5 kg/m², without current pregnancy related complications and having

a singleton pregnancy. Exclusion criteria included, aged <18 years, BMI <18.5kg/m^2, participants with multiple pregnancy, those currently involved in another research study, participants with a history of gastrointestinal, hepatic, renal, vascular or haematological disorders. In addition, participants who have had in vitro fertilisation (IVF) treatment, participants with a history of NTD affected pregnancies and pregnant women with active thyroid disease (e.g., Graves, Hashimoto or thyroiditis) were excluded.

All participants provided written informed consent according to the Declaration of Helsinki. The study was reviewed by the Biomedical Sciences Ethics Filter Committee, Ulster University 15/0041 and approved by Office for Research Ethics Committees (ORECNI) 15/NI/0068 and by WHSCT (WT 14/49). The study was registered at the ClinicalTrials.gov ID: NCT02713009.

Sample size

A sample size of 94 in each treatment group was calculated to provide 90% power at a significance level of $p<0.005$ in vitamin D status. This was based on a previous study by Hollis *et al*, 2011. Based on Hollis *et al.*, 2011 and to account for potential dropouts, (those who discontinue the study, suffer miscarriage or pregnancy complications or poorly comply with the study protocol) the sample size was increased by 25% to give a total of 120 in each treatment group.

Randomisation

Participants were randomised and stratified by BMI using Minim randomisation software, by an independent researcher, and randomly assigned to receive either 10µg or 20µg vitamin D from 12 weeks gestation through to delivery (119 participants were given two tablets, one multivitamin tablet (containing 10µg/vitamin D) plus a 0µg vitamin D (placebo) tablet and 121 participants were given two tablets, one multivitamin tablet (containing 10µg/vitamin D) plus a 10µg vitamin D tablet).

10µg is the current UK recommendation for pregnant women (SCAN, 2016); the multivitamin used in the study contained 10µg. Whereas 20µg was based on a previous study by Holmes et al., 2009, where the vitamin D status of non-supplement using pregnant women with obesity was 25 nmol/L; in order to rise this status to a level of sufficiency (>50nmol/L), it was calculated that 20µg/d was required, as approximately 1µg of vitamin D may increase status by 1.2 nmol/L.

Intervention

The multivitamins (Vitabiotics, Pregnancare®) were supplied from Vitabiotics, and vitamin D_3 and placebo from (Sona® Nutrition). The placebo and vitamin D tablets were matched for size, colour and texture. The tablets were provided to the participants in two batches comprising of weekly pill boxes, by the researcher at their appointments; batch one from week 12 to 28 weeks gestation and batch two from 28 to 40 weeks gestation. Participants were contacted by the researcher twice between 12 and 28 weeks gestation and again between 28 to 34 weeks gestation to check compliance and answer any questions the participant had. Following the second visit

participants returned the pill boxes; all unused tablets were counted and recorded. This procedure was repeated at the third visit and following this, any remaining pill boxes were returned via a free post envelope after the birth. Compliance was defined as supplement consumption > 75%.

Data collection

First antenatal visit

Anthropometry and body composition

At the first antenatal visit around (12 weeks gestation), anthropometric and body composition measurements were taken including height (using a stadiometer), weight, fat mass (kg) and fat free mass (kg) (using TANITA, MC-780MA scale). BMI was calculated as kg/m^2 (World Health Organisation, 2004). All measurements were carried out by trained researchers in a private environment within the clinic setting.

Health and Lifestyle information

All participants completed a Health and Lifestyle Questionnaire at the first antenatal visit, which recorded information on age, social demographics, medication, supplementation use and sun exposure, including sun bed/bathing use and whether participants had been on a sun holiday within the previous 6 months. In addition, details from maternal notes were recorded including weeks gestation, parity, blood pressure, smoking and previous miscarriage by the researcher during the first visit.

Subsequent visits

Participants attended follow-up appointments at 28 and 34 weeks gestation. Anthropometric and body composition measurements were repeated as per the initial visit. Information from maternal notes was recorded including weeks gestation, blood pressure, growth chart for the fetus, routine blood and urine sample results during pregnancy by the researcher at each visit. Infants anthropometric measures at birth (weight, length, head circumference) and other measures relevant to the health status of the mother and child were recorded from maternal notes and paediatric charts after delivery by the researcher.

Dietary intake

At the second trimester visit, all participants completed a validated Food Frequency Questionnaire (FFQ) to assess vitamin D intake from foods (Weir *et al.*, 2016). The FFQ were assessed by portion size of the food and how often they consumed the food based on variety of foods which contribute to dietary vitamin D intake (milk and dairy, cereal products, meat and fish, eggs, cakes and confectionery, other fortified foods/drinks and dietary supplements).

Blood sample analysis

Non-fasting blood samples were collected at 12, 28 and 34-36 weeks gestation by a fully trained phlebotomist and cord blood samples were collected at delivery by the midwife on duty. A total of 20 ml (2x8ml serum tubes and 1x4ml plasma tube) of blood was collected and kept chilled and processed within 3 hours of collection

(centrifugation at 3000 rpm for 15 minutes). Plasma and serum aliquots were stored at -80°C until batch analysis. Stored serum samples were used for vitamin D analysis. This was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) by quantifying and summing 25-hydroxyvitamin D_2 ($25(OH)D_2$) and 25-hydroxyvitamin D_3 ($25(OH)D_3$) to give total 25-hydroxyvitamin D (25(OH)D) concentrations (Chromsystems Instruments and Chemicals GmbH, Gräfelfing, Germany; MassChrom 25-OH-Vitamin D_3/D_2 and API 4000 LC-MS/MS; AB SCIEX, Washington, DC, USA). Vitamin D status was classified into categories of sufficiency according to SACN guidelines and defined as deficient (25(OH)D <25nmol/L); insufficient (25(OH)D 25-50nmol/L) or sufficient (25(OH)D >50nmol/L) (SACN, 2016). Plasma intact parathyroid hormone (PTH) concentrations was measured using a commercially available enzyme-linked immunosorbent assay (MD Biosciences Inc., Minnesota, USA). Samples were batch analysed in the Biochemistry Department of St James's Hospital (Dublin, Ireland).

Serum concentrations of calcium and albumin were measured using an automated clinical chemistry system (Ilab 650 Clinical Chemistry System, WERFEN). Albumin-adjusted calcium was calculated using the following formulae:

$$\text{Adjusted calcium} = \text{measured total calcium} + 0.02(40 - \text{albumin})$$

Or for albumin greater than 45g/L:

$$\text{Adjusted calcium} = \text{measured total calcium} - 0.02(45 - \text{albumin})$$

Statistical analysis

The statistical analyses were performed using SPSS (Statistical Package for the Social Sciences software, Version 22; IBM). Data were assessed for normality using Kolmogorov-Smirnov test. Data were presented as mean ± SD. Intention to treat analysis was conducted by carrying forward the last available measurement e.g., vitamin D status missing at second visit was input with the vitamin D status measured at the baseline visit. Repeated measures ANOVA was used to compare the effect of 10μg and 20μg supplementation, adjusted for age, BMI group, supplement use and season at baseline. Differences between 10 μg and 20μg groups in 25(OH)D were assessed by ANCOVA adjusted for age, BMI, supplement use at baseline and seasons in each trimester. Differences between 10μg and 20μg treatment groups for adjusted calcium and PTH were also assessed by ANCOVA adjusted for age, BMI and supplement use at baseline.

Independent-samples t-test was used to assess differences in maternal characteristics between treatment groups. Chi-Square tests were performed to determine associations between the category of vitamin D sufficiency and other categorical variables including smoking, education level, marital status, parity, medication use, dietary supplement use, recent sun holiday, sunbed/bathing use and season. Bivariate correlations were performed between 25(OH)D concentrations and age, body composition measures, biochemical measures and blood pressure. In addition, multivariate-adjusted multiple regression models were used to assess determinants of vitamin D status. Differences between 10μg and 20μg treatment groups in infants' characteristics were assessed by ANCOVA adjusted for BMI group and categorical variables including mode of delivery, baby weight and gender were assessed by Chi-squared test. Results were considered significant when P value <0.05 in all analyses.

Results

Recruitment

240 pregnant women completed the baseline visit (119 in 10µg group and 121 in 20µg group); samples were available for all but one participant from the 10µg group who withdrew from the study (personal reasons). A total of 74 participants withdrew from the study after baseline (37 in 10µg group and 37 in 20µg group) and the dropout according to BMI category in the 10µg group was n 13 normal weight, n 14 overweight, n 9 obese and in the 20µg group was n 10 normal weight, n 14 overweight, n 14 obese, with no significant difference observed between BMI categories in each treatment group for those who dropped out. A total 158 participants completed the second trimester visit and provided blood samples (78 in 10µg group and 80 in 20µg group); 4 women in each group did not provide samples due to sickness or inability to attend the appointment. At the third trimester visit, 153 participants completed study measurements and provided blood samples (73 in 10µg group and 80 in 20µg group); 9 women in the 10µg group did not provide blood samples (n=5 unable to attend the appointment and n=4 due to preterm delivery). In the 20µg group 4 women did not provide blood samples (n=1 due to sickness, n=1 due to preterm delivery, n=2 due to inability to attend the appointment; details of reasons for withdrawal are explained in Figure 2). Compliance with the intervention was high with no difference between participants in the 10µg and 20µg treatment groups: 92.4% vs. 91.7%, P= 0.856.

Maternal characteristics

A total of 239 pregnant women were included in this analysis, 118 in the 10 µg/d group and 121 in the 20µg/d group. There were no significant differences at baseline for anthropometric characteristics, weeks' gestation, blood pressure, education, medication use, use of supplements containing vitamin D, vitamin D dietary intake and season of enrolment between the 10µg and 20µg treatment groups (Table1).

Infants characteristics

A total of 164 infants (81 in the 10µg group and 83 in the 20µg group) were born to mothers who completed the intervention. At delivery there were no statistical differences in gestational age or mode of delivery between the 10µg and 20µg treatment groups. Nor were there any differences between birth weight, length, apgar scores or gender in infants born in each treatment group. Infants born to mothers in the 10µg group had significantly lower head circumference compared to infants born to mothers in the 20µg group (34.9 ± 1.5 vs. 35.7 ± 2.7 cm, P= 0.020) (Table 6).

Maternal vitamin D status

Maternal 25(OH)D concentrations in each trimester are shown in Table 2. Maternal 25(OH)D concentrations increased from the 1st trimester to 3rd trimester in both the 10µg and 20µg groups, with a higher increase in the 20µg group (P<0.0001). The mean ± SD increase was 17.1 ± 24.7 and 28.8 ± 33.3 nmol/L, P=0.002, in the 10µg and 20µg groups respectively, after adjustment for age, BMI, vitamin D supplement use at baseline and season.

Maternal 25(OH)D concentrations at the baseline visit were not significantly different between the 10µg and 20µg groups; 52.2 ± 22.9 vs. 52.0 ± 20.5 nmol/L, *P*=0.655. At the 2nd and 3rd trimester visits, 25(OH)D concentrations were significantly lower in the 10µg group compared with the 20µg group (trimester 2: 62.8 ± 26.3 vs. 73.6 ± 31.6 nmol/L, *P*= 0.005; trimester 3: 69.3 ± 30.7 vs. 80.8 ± 37.1 nmol/L, *P*= 0.012) after adjustment for age, BMI, vitamin D supplement use at baseline and season.

Maternal 25(OH)D concentrations in the 1st trimester (baseline) were not significantly different between the 10µg and 20µg groups among normal weight, overweight and obese women (Table 3). In the 2nd and 3rd trimesters 25(OH)D concentrations of normal weight women were significantly lower in the 10µg group compared with the 20µg group (64.3 ± 25.8 vs. 84.1 ± 30.4 nmol/L, *P*= 0.012; 71.3 ± 30.4 vs. 94.3 ± 36.2 nmol/L, *P*= 0.018) after adjustment for age, vitamin D supplement use at baseline and season. There were no observed differences in 25(OH)D concentrations in the 2nd and 3rd trimesters between treatment groups in either overweight or obese women.

Maternal 25(OH)D concentrations across BMI categories in each trimester for both the 10µg and 20µg groups are presented in Table 4. There were no statistical significant differences in maternal 25(OH)D concentrations across BMI categories in any trimester in the 10µg group. Whereas, in the 20µg group, maternal 25(OH)D concentrations were significantly lower in pregnant women with obesity compared with women of normal weight in the 2nd trimester and 3rd trimester (64.7 ± 30.9 vs. 84.1 ± 30.4 nmol/L, *P*=0.037; 67.7 ± 34.5 vs. 94.3 ± 36.2 nmol/L, *P*=0.014) after adjustment for age, vitamin D supplement use at baseline and season.

The prevalence of insufficiency (<50 nmol/L) and sufficiency (≥50 nmol/L) in both the 10µg and 20µg groups in each trimester are presented in Table 5. There were no

differences in the levels of insufficiency/sufficiency between the 10µg and 20µg groups in any trimester; nor was there a difference in insufficiency/sufficiency when assessed by BMI category.

Figure 3A presents 25(OH)D concentrations in each trimester for the 10µg and 20 µg groups, split by those who had a baseline 25(OH)D concentration <50nmol/L vs. ≥50nmol/L. Pregnant women who started pregnancy with an insufficient 25(OH)D concentration remained insufficient in 25(OH)D throughout pregnancy in the 10µg group however, in the 20µg group women who started pregnancy classed as insufficient reached 25(OH)D sufficiency in the 2nd and 3rd trimesters. Whereas, pregnant women who started pregnancy with a sufficient 25(OH)D concentration, maintained sufficiency throughout pregnancy in both the 10µg and 20µg groups. When split by BMI category, similar findings were obtained in the normal weight, overweight and obese categories.

Cord vitamin D status

Sixty-two cord blood samples were collected (31 samples in each of the 10 and 20µg groups) (Table 2). Cord blood 25(OH)D concentrations were not statistically different between infants born to mothers in the 10µg vs. 20µg groups (35.5 ± 15.1 vs. 42.2 ± 19.7 nmol/L, P=0.208). Cord blood 25(OH)D concentrations were positively correlated with maternal 25(OH)D concentrations in the 1st, 2nd and 3rd trimesters (r=552, P<0.0001; r=585, P<0.0001; r=784, P<0.0001) respectively. There was no statistical difference in levels of cord blood insufficiency/sufficiency between the 10µg and 20µg groups (Table 5). Furthermore, cord blood 25(OH)D concentrations were significantly lower in infants born to mothers with obesity in the 20µg group

compared to infants born to mothers of normal weight (28.8 ± 16.9 vs. 52.1 ± 17.3 nmol/L, *P*=0.027) after adjustment for season of delivery (Table 4).

When the mother started pregnancy with an insufficient status, corresponding mean cord blood 25(OH)D concentrations were found to be insufficient in infants born to mothers in both the 10µg and 20µg groups (31.2 ± 17.0 vs. 26.3 ± 12.0 nmol/L, *P*=0.461) respectively. Whereas, when the mother had a sufficient status at the beginning of pregnancy, sufficient cord blood 25(OH)D concentrations (based on mean) were found in infants born to mothers in 20µg group but not in infants born to mothers in 10µg group (53.7 ± 15.9 vs. 36.8 ± 14.7 nmol/L, *P*=0.001) respectively, (Figure 3 A).

Similar trends which were dependent on maternal insufficiency/sufficiency in early pregnancy were observed in the cord blood 25(OH)D concentrations of infants born to normal weight and overweight pregnant women (Figures 3 B, C). However, in women with obesity who started pregnancy with an insufficient status, cord blood results were found to be classified as deficient regardless of 10µg vs. 20µg treatment (19.4 ± 20.2 vs. 19.5 ± 9.4 nmol/L, *P*= 0.992). In contrast, obese women who started pregnancy as sufficient had cord blood results above deficiency and within the insufficiency in both the 10µg vs. 20µg treatment groups (Figure 3 D).

When 3rd trimester maternal 25(OH)D concentrations were related to cord blood 25(OH)D concentrations, women who had an insufficient status in late pregnancy were found to have cord blood levels classified as deficient, regardless of 10µg vs. 20µg treatment (12.7 ± 2.6 vs. 23.2 ± 16.7 nmol/L, *P*=0.460). In contrast, when 3rd trimester maternal 25(OH)D concentrations were sufficient, corresponding cord blood 25(OH)D concentrations were classified as insufficient (and out of the deficiency

category) in both the 10µg vs. 20µg treatment groups (37.1 ± 14.3 vs. 44.3 ± 19.2 nmol/L, *P*=0.118).

There was no difference in the percentage placental transfer of maternal 25(OH)D concentrations in the 3rd trimester to the cord blood 25(OH)D concentration between the 10µg and 20µg groups after adjustment for season of delivery (41% vs.42%, *P*=0.807) respectively; nor were there any differences in placental transfer of 25(OH)D between the 10µg and 20µg groups among pregnant women of normal weight, overweight and obesity.

Pregnant women with obesity in the 10µg group who had an insufficient 25(OH)D concentration (<50 nmol/L) in the 1st trimester transferred a lower 25(OH)D concentration to their infants compared with women who received 20µg vitamin D (21.5 % vs. 32.8%, *P*=0.046).

Vitamin D supplement use at baseline and vitamin D dietary intake assessed in the 2nd trimester from a validated vitamin D FFQ were found to be significant predictors of maternal 25(OH)D percentage change (from 1st to 3rd trimester) in adjusted regression models (β= 0.181, *P*=0.005; β=-0.144, *P*=0.024) respectively.

Maternal adjusted calcium and PTH concentrations are shown in Table 2. There were no differences in these blood biomarkers over the time of the intervention between the 10µg and 20µg treatment groups after adjustment for age, BMI and vitamin D supplement use at baseline. There were no associations found between maternal adjusted calcium concentrations and maternal 25(OH)D concentrations in any trimester. In the 1st and 3rd trimesters maternal PTH concentrations were negatively correlated with maternal 25(OH)D concentrations (*r*=-0.343, *P*<0.0001; *r*=-0.130, *P*=0.045). Maternal 25(OH)D concentrations in the 3rd trimester positively correlated

with infant birth weight and head circumference (r=0.157, *P*=0.45; r=0.187, *P*=0.017) respectively.

Discussion

There is no global consensus as to what constitutes adequate vitamin D supplementation during pregnancy; the current UK recommendation of 10µg/d of vitamin D for all pregnant and lactating women has been set with the aim of preventing maternal and infant deficiency (SACN, 2016). When we assessed the vitamin D status at the group level of 239 pregnant women, both 10µg and 20µg of vitamin D/d during pregnancy seemed to increase vitamin D status to a sufficient status (≥50 nmol/L) for the duration of pregnancy. A recent study by O'Callaghan *et al.,* 2018 compared 3 supplement groups of vitamin D, placebo, 10µg and 20 µg/d and reported similar findings. Although, in our study, we showed that the increase in vitamin D status was significantly higher in the 20µg group compared with the 10µg group. There was no observed difference in the related cord blood vitamin D status between the 10µg and 20µg treatment groups, both were classed as being an insufficient status (<50 nmol/L). However, the definition of adequate or optimal vitamin D status for maternal health is controversial, and currently there are no known requirements or thresholds for 25(OH) D concentrations in cord blood samples indicative of improved infant health outcomes.

Previous studies have shown a negative association between maternal BMI and maternal vitamin D status (Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.,* 2013) however, based on current knowledge no studies have assessed the effect of vitamin D supplementation on pregnant women in different BMI categories. Therefore, this study shows the treatment effect of vitamin D supplementation of both 10µg/d and 20 µg/d on pregnant women across different BMI categories. Normal weight pregnant women in the 20µg/d group had significantly increased maternal and cord vitamin D status post intervention compared to normal weight pregnant women in 10µg/d group. There were no observed differences in vitamin D status in the 2nd and 3rd trimesters

between treatment groups in either overweight or obese women. This shows that pregnant women of normal weight had a higher response to a higher dose (20µg) of vitamin D compared to pregnant women who were overweight and obese. In the 20µg supplementation group, pregnant women who were classified as obese had significantly lower vitamin D status in the 2nd and 3rd trimesters and in corresponding cord blood compared with pregnant women of normal weight; no significant difference was observed in vitamin D status in the 1st trimester. This observed difference could be related to sequestration of vitamin D in adipose tissue, with obese participants typically carrying more adipose tissue than their normal weight or overweight counterparts. Other physiological changes during pregnancy including weight gain, haemodilution and volumetric dilution which are also postulated to influence vitamin D status (Drincic *et al.,* 2012). Haemodilution and weight gain during pregnancy are thought to have a similar effect on vitamin D status in pregnant women across different BMI categories (Karlsson *et al.,* 2015), however a dearth of knowledge remains on this issue.

Women who commenced pregnancy with insufficient vitamin D status failed to reach sufficiency throughout pregnancy in the 10µg/d group, which shows that the current recommendation is inadequate to reach a sufficient status in those who start pregnancy with a low vitamin D status. In our study we showed that 41.5% and 48.8% of women had an insufficient status on entering pregnancy in the 10µg and 20µg groups, and even with reported supplement use, 40.8% and 57.6% of women in the 10µg and 20µg groups, were still classified as insufficient. It has been noted previously that over 90% of pregnant women living in the same latitude, had an insufficient vitamin D status even with supplement use (Holmes *et al.,* 2009). Given our large population of pregnant women, our findings can be considered reflective of the current vitamin D

status of women in early pregnancy, who, even when adherent to the current recommendation of 10µg/d, may not reach a sufficient vitamin D status at any stage during pregnancy. Although, we have shown that as per the aim of the current SACN recommendation, 10µg/d of vitamin D supplementation during pregnancy is enough to prevent pregnant women and their infant from vitamin D deficiency (<25 nmol/L), however is not enough to ensure maternal or cord sufficiency. Pregnant women who started pregnancy with a sufficient vitamin D status remained sufficient throughout pregnancy in both the10µg and 20µg groups. Cord blood vitamin D concentrations only reached a sufficient status in pregnant women taking 20µg/d and not in those women taking 10µg/d. Whilst these findings may be viewed as encouraging for the 10µg SACN supplementation strategy, over 45% of women in this cohort would not reach these levels of maternal sufficiency, as they started pregnancy with an insufficient status. In addition, even when starting pregnancy with a sufficient status, the 10µg SACN supplementation strategy is inadequate to achieve a sufficient 25(OH) cord concentration.

Previous studies have reported that 10µg/d of vitamin D may prevent pregnant women from vitamin D deficiency (Hollis *et al.,* 2011; Asemi *et al.,* 2013), and whilst this may have been the case in these studies, many factors can affect the measurement of vitamin D status which may account for some of the variance observed between studies even when the same supplementation dose is used, these include latitude, vitamin D status at the baseline of the study, dietary intake and study design.

Similar to finding at the total group level, findings in normal weight and overweight pregnant women showed that 10µg/d of vitamin D supplementation during pregnancy is enough to prevent pregnant women and their infant from vitamin D deficiency (<25 nmol/L) however is not enough to ensure maternal or cord sufficiency. Pregnant

women with obesity who started pregnancy with an insufficient vitamin D status had deficient (<25 nmol/L) cord blood concentrations in both the 10µg and 20µg groups despite mothers having a sufficient vitamin D status in 3rd trimester, this was not observed in mothers who were either normal weight or overweight. This result supported the previous findings that neonates of obese mothers have significantly lower cord vitamin D status compared to neonates of normal weight mothers (Bodnar *et al.,* 2007; Karras *et al.,* 2013) despite no differences in maternal 25(OH)D (Josefson et al., 2013). This result again reinforces our finding that the current recommendation of 10µg vitamin D during pregnancy is inadequate particularly based on the high prevalence of vitamin D insufficiency and on the current high prevalence of maternal obesity in UK populations. The potentially putting infants born to mothers with obesity at high risk for vitamin D deficiency and poor in-utero bone development (Weinert *et al.,* 2015; Leffelaar *et al.,* 2010; Robinson *et al.,* 2011).

Cord blood vitamin D concentrations are usually 60-80% of maternal vitamin D values at delivery (Hollis *et al.,* 1984; Vieth *et al.,* 2013). However, the current study found cord vitamin D concentrations to be 41% and 42% of maternal vitamin D in late pregnancy, with no difference between the treatment groups. We did find that the percentage of placental transfer was significantly lower among pregnant women with obesity who started pregnancy with insufficient vitamin D status and received 10µg/d compared with pregnant women with obesity who started pregnancy also with insufficient vitamin D status and received 20µg/d (21.5% and 32.8% P=0.046).

The overall mean vitamin D status for both treatment groups at baseline was considered sufficient, yet there was still a high prevalence of vitamin D insufficiency; similar to findings from a recent study of 144 pregnant women in Cork, Ireland (O'Callaghan *et al.,* 2018). In our study, after supplementation in the 3rd trimester the

prevalence of vitamin D insufficiency dropped from 41.5% and 48.8% to 27.1% and 24.8% in the 10µg and 20µg groups respectively, compared to 5% and 2% in O'Callaghan *et al.,* 2018 study. The higher prevalence of insufficiency in the 3rd trimester observed in the current study may be due to a number of reasons, firstly participants in current study received exactly 10µg or 20µg daily, which is different to the O'Callaghan *et al.,* 2018 study, as they allowed participants to continue with their own antenatal supplement regime, allowing for possible variation in the multivitamin type and vitamin D amount within supplements. In addition, we had equal numbers with BMI categories and within treatment groups whereas O'Callaghan *et al.,* 2018 had more normal weight women in the 20µg group and overweight in the 10µg group. We demonstrated that normal weight women had a higher response in vitamin D change in the 20µg group compared to the overweight and obese on this supplement dose, therefore the high numbers of normal weight in the 20µg treatment group in the O'Callaghan *et al.,* 2018 study may have contributed to their higher status and lower prevalence of insufficiency.

To account for seasonal variation, recruitment of our participants was ongoing throughout the year, across all seasons, winter (October-March) and summer (April-September). All statistical analysis was adjusted for season. A previous UK study conducted in across different cities (Southampton, Oxford and Sheffield) of 1134 pregnant women showed that a higher dose (25µg) of vitamin D was enough to prevent seasonal decline of maternal vitamin D status and maintain concentrations (≥ 50 nmol/L) however, the mean maternal BMI at baseline considered as normal which not reflect other maternal BMI groups also cord vitamin D status was not assessed to ensure adequate vitamin D supplement to prevent infant vitamin D deficiency (Cooper *et al.,* 2016).

Maternal adjusted calcium and parathyroid hormone concentrations remained unchanged throughout pregnancy in both the 10µg and 20µg groups with no differences observed between treatment groups. A negative correlation between maternal PTH and maternal vitamin D status in the 1st and 3rd trimester was observed this is expected with vitamin D metabolism; O'Callaghan *et al.,* 2018, reported similar findings.

Infants characteristics were not different between the 10µg and 20µg groups however; head circumference was higher in infants born to mothers in the 20µg group compared to infants born to mothers in the 10µg group. Maternal vitamin D status in the 3rd trimester was positively correlated with infant weight and head circumference, similar results were observed by Gernand *et al.,* 2013, which might explain that higher vitamin D status during pregnancy associated with better growth of infant.

This study has a number of strengths, including that vitamin D status was assessed by quantifying 25(OH)D concentrations using the gold-standard method of LC-MS/MS (Institute of Medicine, 2010). Furthermore, BMI and vitamin D were measured at the same time to accurately assess the association between body weight and vitamin D status, with equal numbers of women across the BMI categories. Moreover, the blood sampling for vitamin D measurement occurred across all seasons allowing for the impact of seasonal variation to be investigated. Vitamin D was assessed at three time points throughout pregnancy and in cord blood samples at delivery. Dietary vitamin D intake was assessed using a validated vitamin D FFQ. In addition, there was a large sample size, and a high rate of compliance in both intervention groups. There was no difference in the maternal characteristics and maternal vitamin D status at baseline, which help to summarise the results of the intervention without any confounders. There are some limitations in this study, including the relatively high dropout rate with

30% of pregnant women failing to complete the study however, the power calculation had accounted for a high dropout rate and differences were still observed in the current study. In addition, only a small number of cord blood samples were available.

Conclusion

These findings demonstrate that the current recommendation of 10µg/d is inadequate to ensure sufficient vitamin D status throughout pregnancy for women living in Northern Ireland, particularly for those who start pregnancy with a low vitamin D status. This risk is further increased in obese women who enter pregnancy with a low vitamin D status. Cord blood from obese women who enter pregnancy with a low vitamin D status was vitamin D deficient, potentially putting infants at high risk for vitamin D deficiency and poor in-utero bone development. This research highlights the need for revised maternal policy on vitamin D recommendations during pregnancy, particularly considering our findings on BMI and the implications for related cord blood and infant outcomes.

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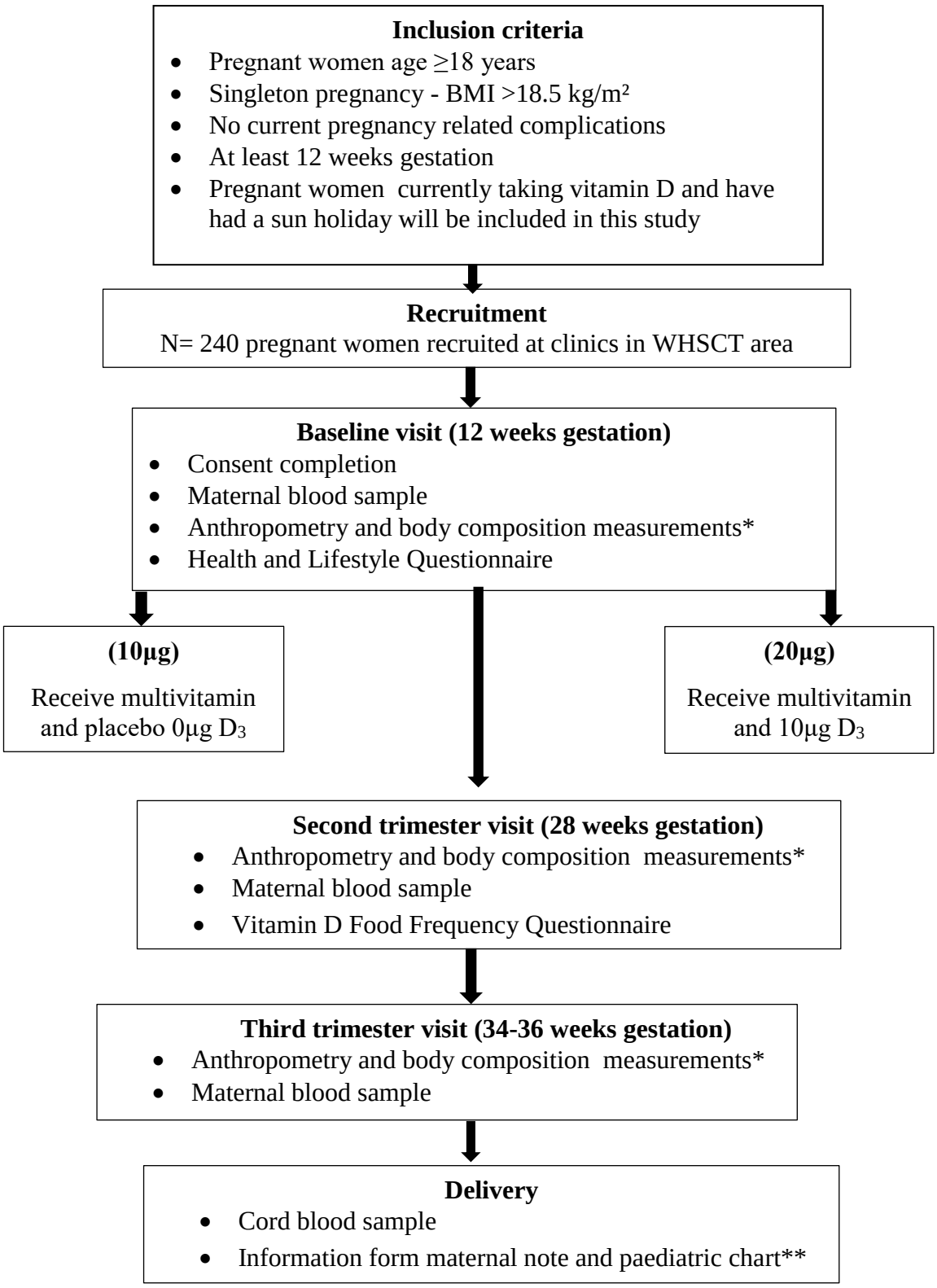

Figure 1: Flow diagram of study design

* Anthropometric and body composition measurements were taken and including height (using a stadiometer), weight, fat mass (kg) and fat free mass (kg) (using TANITA, MC-780MA scale).

** Infants anthropometric measures at birth (weight, length, head circumference) and other measures relevant to the health status of the mother and child. (WHSCT) Western Health and Social Care Trust.

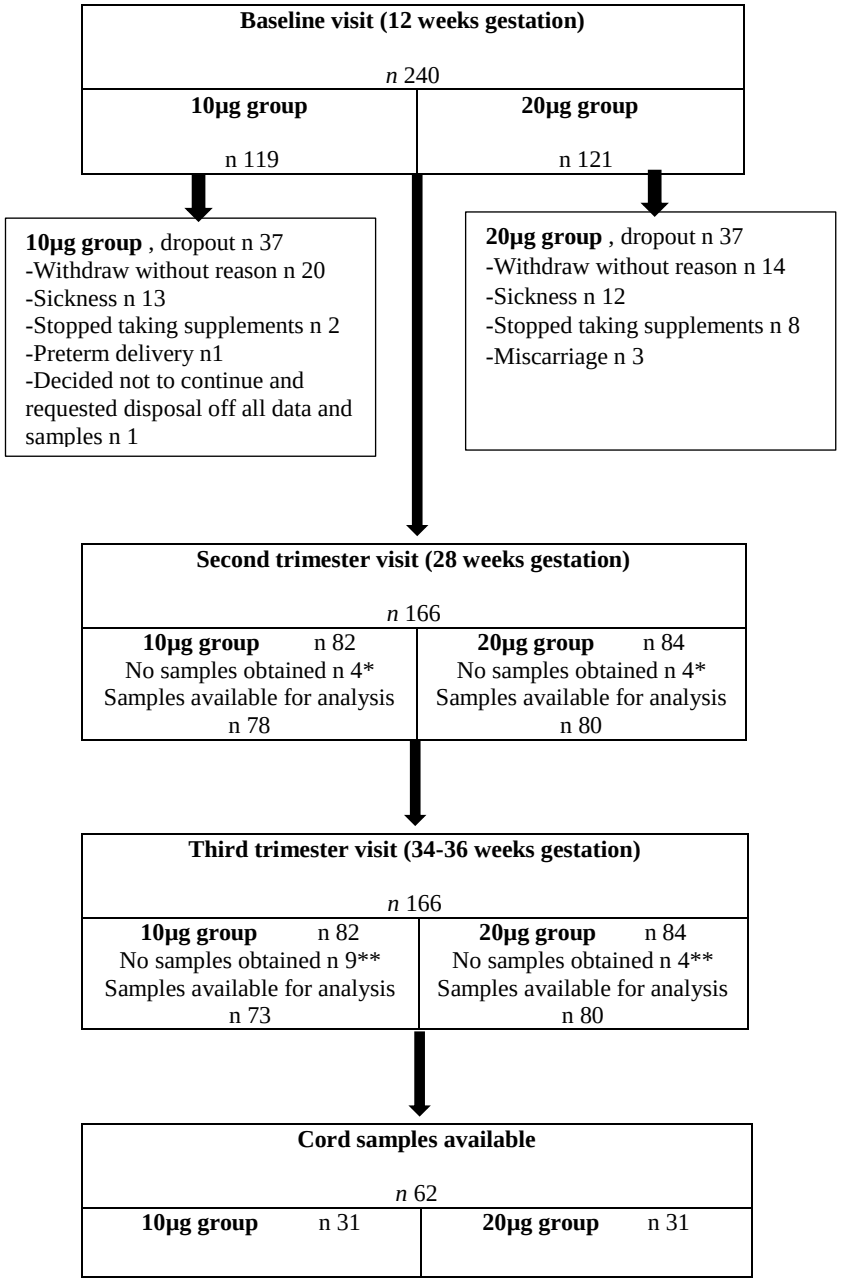

Figure 2. Flow diagram of study participants

*Sickness (n 2 in 10µg group, n 2 in 20µg group), unable to make appointment (n 2 in 10µg group, n 2 in 20µg group).

**Sickness (n 1 in 20µg group), unable to make appointment (n 5 in 10µg group, n 2 in 20µg group), preterm delivery (n 4 in 10µg group, n 1 in 20µg group).

Table 1: Maternal characteristics in 10 µg and 20 µg treatment groups.

	10 µg	**20 µg**	***P***
	n 118	**n 121**	
Age (yrs)	29.7 ± 5.1	29.5 ± 5.5	0.810
Weight (kg)	74.7 ± 15.9	74.4 ± 15.9	0.880
Height (m)	1.63 ± 0.06	1.63 ± 0.06	0.703
BMI (kg/m^2)	28.1 ± 5.7	27.8 ± 5.4	0.678
Fat (%)	34.0 ± 6.4	34.0 ± 7.3	0.982
Fat mass (kg)	26.5 ± 10.5	26.6 ± 10.6	0.928
Fat free mass (kg)	48.2 ± 6.7	47.8 ± 6.8	0.617
Fat mass index (kg/m^2)	9.9 ± 3.8	9.9 ± 3.8	0.944
Fat free mass index (kg/m^2)	18.3 ± 3.0	17.9 ± 2.1	0.191
Gestational weeks (wk)	13.0 ± 1.4	12.8 ± 1.4	0.363
Blood pressure (mmHg)			
Systolic	119.3 ± 11.1	120.5 ± 11.6	0.415
Diastolic	71.8 ± 8.5	72.7 ± 9.0	0.403
Smoker n (%)	16 (13.7)	14 (11.7)	0.642
Parity n(%)			
0	53 (45.3)	43 (36.1)	0.175
1	34 (29.1)	48 (40.3)	
2$^+$	30 (25.6)	28 (23.5)	
Previous miscarriage n(%)	41 (34.7)	30 (24.8)	0.092
Education level n(%)			
Secondary	38 (32.8)	36 (30.8)	0.939
Diploma	18 (15.5)	23 (19.7)	
Degree	38 (32.8)	38 (32.5)	
Postgraduate	16 (13.8)	14 (12.0)	
other	6 (5.2)	6 (5.1)	
Marital status n(%)			
Married	60 (50.8)	62 (51.2)	0.952
Unmarried	58 (49.2)	59 (48.8)	
Regular medication n(%)	15 (12.7)	8 (6.6)	0.110
Medical illnesses n(%)	13 (11.0)	9 (7.4)	0.339
Vitamin D supplement use n(%)			
User	66 (55.9)	81 (66.9)	0.080
Non-user	52 (44.1)	40 (33.1)	
Sun holiday past 6 months n(%)	20 (16.9)	23 (19.2)	0.657
Sun bathing/sunbed last month n(%)	7 (6.0)	11 (9.2)	0.355
Season at enrolment n (%)			
Winter (October-March)	61 (47.7)	67 (52.3)	0.569
Summer (April-September)	57 (51.4)	54 (48.6)	
Vitamin D dietary intake (µg/d)*	4.3 ± 2.2	4.3 ± 2.8	0.978

Data are presented as Mean ± SD or n (%). BMI – body mass index (kg/m^2) was calculated as weight (kg) divided by height^2 (m^2) (WHO). Fat mass index (kg/m^2) Fat mass (kg) divided by height^2 (m^2). Fat free mass index (kg/m^2) Fat free mass (kg) divided by height^2 (m^2). *Vitamin D dietary intake assessed form validated vitamin D FFQ. Differences between group 10 µg and 20 µg groups were assessed by Independent sample t-test or Chi-squared test as appropriate, ($P< 0.05$) considered significant

Table 2: Intention to treat analysis of pregnant women on maternal 25(OH)D, adjusted calcium and PTH and cord 25(OH)D, cord adjusted calcium.

	1st trimester			2nd trimester			3rd trimester				Cord		
	10 µg n 118	20 µg n 121	*P*	10 µg n 118	20 µg n 121	*P*	10 µg n 118	20 µg n 121	*P*	***P****	10 µg n 31	20 µg n 31	*P*
25(OH)D (nmol/L)	52.2 ± 22.9	52.0 ± 20.5	0.655	62.8 ± 26.3	73.6 ± 31.6	0.005	69.3 ± 30.7	80.8 ± 37.1	0.012	<0.0001	35.5 ± 15.1	42.2 ± 19.7	0.208
Adjusted calcium (mmol/L)	2.29 ± 0.10	2.30 ± 0.11	0.614	2.27 ± 0.11	2.29 ± 0.08	0.127	2.27 ± 0.11	2.28 ± 0.10	0.769	0.580	2.6 ± 0.23	2.6 ± 0.25	0.618
Parathyroid hormone (PTH)	21.2 ± 7.8	21.9 ± 9.0	0.441	23.4 ± 9.1	22.8 ± 8.2	0.643	23.8 ± 9.3	23.5 ± 9.0	0.789	0.296			

Data presented as mean ± SD. *P* Differences between 10 µg and 20 µg groups in 25(OH)D were assessed by ANCOVA adjusted age, BMI, supplement use at baseline and seasons in each trimester and cord for the adjusted calcium and (PTH) only adjusted age, BMI and supplement use at baseline, ($P< 0.05$) considered significant. *P** obtained from repeated measure ANOVA, comparing the effect of treatment group adjustment for age, BMI group, supplement use at baseline and season at baseline. ($P< 0.05$) considered significant.

Table 3: Differences in vitamin D status between 10 µg and 20 µg groups in each BMI category.

	1st trimester			2nd trimester			3rd trimester			Cord		
	10 µg	20 µg	***P***	10 µg	20 µg	***P***	10 µg	20 µg	***P***	10 µg	20 µg	***P***
25(OH)D (nmol/L)												
ALL	52.2± 22.9	52.0 ± 20.5	0.671	62.8 ± 26.3	73.6± 31.6	0.006	69.3 ± 30.7	80.8 ± 37.1	0.012	35.5 ± 15.1	42.2 ± 19.7	0.184
Normal weight	55.9 ± 26.3	59.2 ± 19.8	0.987	64.3 ± 25.8	84.1 ± 30.4	0.012	71.3 ± 30.4	94.3 ± 36.2	0.018	34.9 ± 14.5	52.1 ± 17.3	0.030
Overweight	51.0 ± 22.2	48.7 ± 18.7	0.697	64.9 ± 27.7	71.7 ± 31.2	0.267	69.7 ± 29.5	80.0 ± 36.4	0.136	37.5 ± 8.5	40.1 ± 19.3	0.540
Obese	49.7 ± 19.7	48.0 ± 21.2	0.711	59.2 ± 25.7	64.7 ± 30.9	0.402	66.9 ± 32.7	67.7 ± 34.5	0.739	34.5 ± 20.3	28.8 ± 16.9	0.942

Data presented as mean ± SD. *P* Differences between 10 µg and 20 µg groups were assessed by ANCOVA adjusted Age, supplement use at baseline and seasons in each trimester, ($P< 0.05$) considered significant.

Table 4: Maternal and cord 25(OH)D concentrations across BMI categories in each trimester.

	10 µg					20 µg				
	All	Normal weight	Overweight	Obese	*P*	All	Normal weight	Overweight	Obese	*P*
25(OH)D nmol/L										
1st trimester	**n 118**	**n 39**	**n 39**	**n 40**		**n 121**	**n 41**	**n 40**	**n 40**	
	52.2 ± 22.9*	55.9 ± 26.3*	51.0 ± 22.2*	49.7 ± 19.7*	0.358	52.0 ± 20.5*	59.2 ± 19.8*	48.7 ± 18.7*	48.0 ± 21.2*	0.072
2nd trimester	**n 118**	**n 39**	**n 39**	**n 40**		**n 121**	**n 41**	**n 40**	**n 40**	
	62.8 ± 26.3#	64.3 ± 25.8	64.9 ± 27.7	59.2 ± 25.7	0.548	73.6 ± 31.6#	84.1 ± 30.4a#	71.7 ± 31.2#	64.7 ± 30.9b#	0.037
3rd trimester	**n 118**	**n 39**	**n 39**	**n 40**		**n 121**	**n 41**	**n 40**	**n 40**	
	69.3 ± 30.7#	71.3 ± 30.4#	69.7 ± 29.5#	66.9 ± 32.7#	0.692	80.8 ± 37.1#	94.3 ± 36.2a#	80.0 ± 36.4#	67.7 ± 34.5b#	0.014
Cord	**n 31**	**n 11**	**n 9**	**n 11**		**n 31**	**n 13**	**n 10**	**n 8**	
	35.5 ± 15.1	34.9 ± 14.5	37.5 ± 8.5	34.5 ± 20.3	0.609	42.2 ± 19.7	52.1 ± 17.3a	40.1 ± 19.3	28.8 ± 16.9b	0.027
*P**	<0.0001	0.037	0.005	0.029		<0.0001	<0.0001	<0.0001	0.025	

Data presented as mean ± SD. *P* Differences between the BMI categories were assessed by ANCOVA adjustments for age, supplement use at baseline and seasons in each trimester. In cord were assessed by ANCOVA adjustments for season of delivery ($P < 0.05$) considered significant. *P** Differences between 25(OH)D in each trimester were assessed by ANCOVA adjusted for age, supplement use at baseline, BMI group and season at baseline and for each category of BMI adjustments for age, supplement use at baseline and season at baseline. Different superscript letters are significantly different between BMI categories, different superscript simples are significantly different between trimesters within each BMI category.

Table 5: Prevalence of maternal 25(OH)D insufficiency and sufficiency in each trimester and in cord for 10µg and 20µg groups.

	1st trimester			2nd trimester			3rd trimester			Cord		
	10 µg	20 µg	***P***	10 µg	20 µg	***P***	10 µg	20 µg	***P***	10 µg	20 µg	***P***
All												
Insufficient <50 nmol/L	49 (41.5)	59 (48.8)	0.261	38 (32.2)	31 (25.6)	0.261	32 (27.1)	30 (24.8)	0.682	24 (77.4)	17 (54.8)	0.060
Sufficient >50 nmol/L	69 (58.5)	62 (51.2)		80 (67.8)	90 (74.4)		86 (72.9)	91 (75.2)		7 (22.6)	14 (54.2)	
Normal weight												
Insufficient <50 nmol/L	14 (35.9)	14 (34.1)	0.870	13 (33.3)	6 (14.6)	0.049	10 (25.6)	6 (14.6)	0.219	9 (81.8)	5 (38.5)	0.032
Sufficient >50 nmol/L	25 (64.1)	27 (65.9)		26 (66.7)	35 (85.4)		29 (74.4)	35 (85.4)		2 (18.2)	8 (61.5)	
Overweight												
Insufficient <50 nmol/L	17 (43.6)	22 (55.0)	0.311	11 (28.2)	11 (27.5)	0.944	10 (25.6)	10 (25.0)	0.948	8 (88.9)	6 (60)	0.153
Sufficient >50 nmol/L	22 (56.4)	18 (45.0)		28 (71.8)	29 (72.5)		29 (74.4)	30 (75.0)		1 (11.1)	4 (40)	
Obese												
Insufficient <50 nmol/L	18 (45.0)	23 (57.5)	0.263	14 (35.0)	28 (35.0)	1.000	12 (30.0)	14 (35.0)	0.633	7 (63.6)	6 (75)	0.599
Sufficient >50 nmol/L	22 (55.0)	17 (42.5)		26 (65.0)	26 (65.0)		28 (70.0)	26 (65.0)		4 (36.4)	2 (25)	

Data are presented as n (%). Differences in maternal classifications of insufficiency and sufficiency between 10 µg and 20 µg groups were assessed by Chi-squared test, ($P< 0.05$) considered significant.

Table 6: Infants characteristics born to mothers who completed the intervention.

	10 µg n 81	**20 µg** n 83	***P***
Gestation at delivery (wk)	39.6 ± 2.1	39.8 ± 1.6	0.528
Mode of delivery			
Vaginal delivery	41 (50.6)	38 (45.8)	0.550
Caesarean section	33 (40.7)	36 (43.4)	
Forceps	6 (7.4)	5 (6.0)	
Vacuum	1 (1.2)	4 (4.8)	
Apgar			
1 minute*	8.4 ± 1.4	8.5 ± 1.1	0.678
5 minutes*	8.9 ± 1.0	9.0 ± 0.3	0.479
Anthropometric			
Baby weight (gm)	3492.7 ± 487.7	3628.9 ± 559.6	0.092
Low birth weight n (%)	3 (3.7)	3 (3.6)	0.500
Normal birth weight n (%)	67 (82.7)	63 (75.9)	
Macrosomia n (%)	11 (13.6)	17 (20.5)	
Birth length (cm)*	52.5 ± 3.3	52.9 ± 3.5	0.378
Head circumference (cm)*	34.9 ± 1.5	35.7 ± 2.7	0.020
Gender			
Male	37 (45.7)	43 (51.8)	0.432
Female	44 (54.3)	40 (48.2)	

Data are presented as Mean ± SD or n (%).

Apgar score is a test generally done at one and five minutes after birth to assess the health of new born children immediately after birth, Scores 7 and above are generally normal, 4 to 6 fairly low, and 3 and below are generally regarded as critically low. Birth weight defined by WHO- Low birth weight <2500g and macrosomia >4000g. *P* Differences between 10 µg and 20 µg groups were assessed by ANCOVA adjusted for BMI group or Chi-squared test as appropriate, (P< 0.05) considered significant. * Only n 81 of infant measures in control group available for analysis.

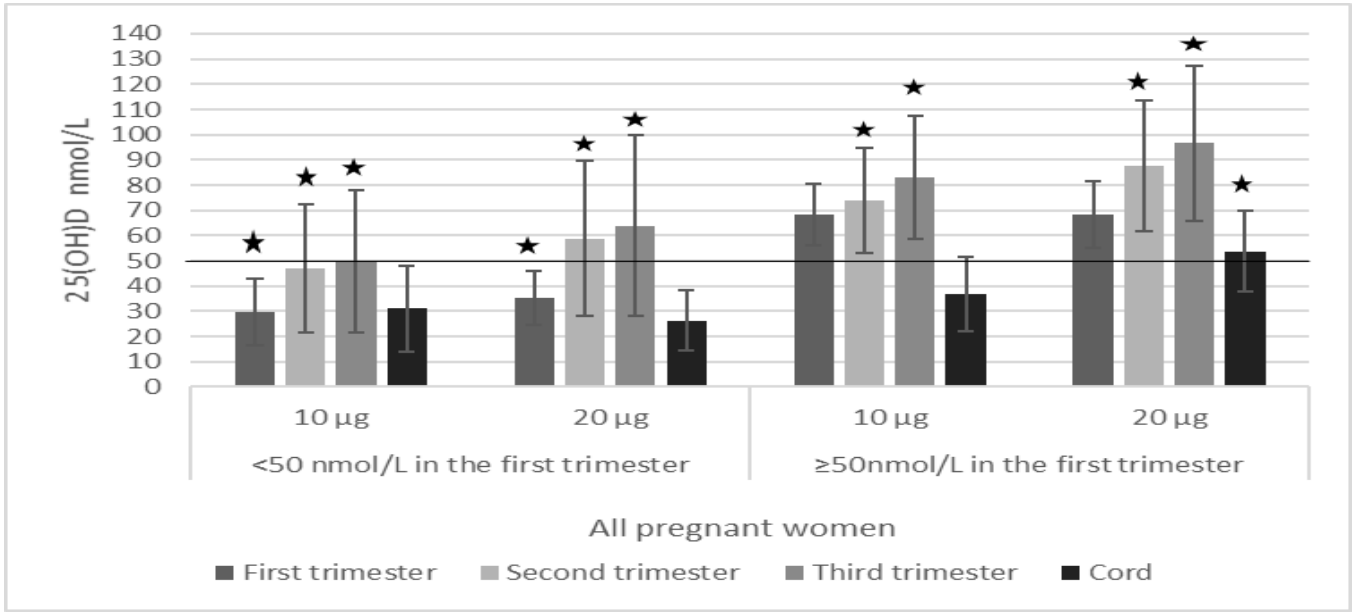

Figure 3 A. 25(OH)D concentrations in 10µg and 20µg groups in all pregnant women throughout pregnancy and in cord for those who started pregnancy with insufficient 25(OH)D concentrations <50 nmol/L and sufficient 25(OH)D concentrations ≥50 nmol/L.★Indicated to significant difference *P* (<0.05) between 10µg and 20µg groups who started pregnancy with insufficient (<50 nmol/L) and between 10µg and 20µg groups who started pregnancy with sufficient (≥50 nmol/L) were assessed by independent t-test.

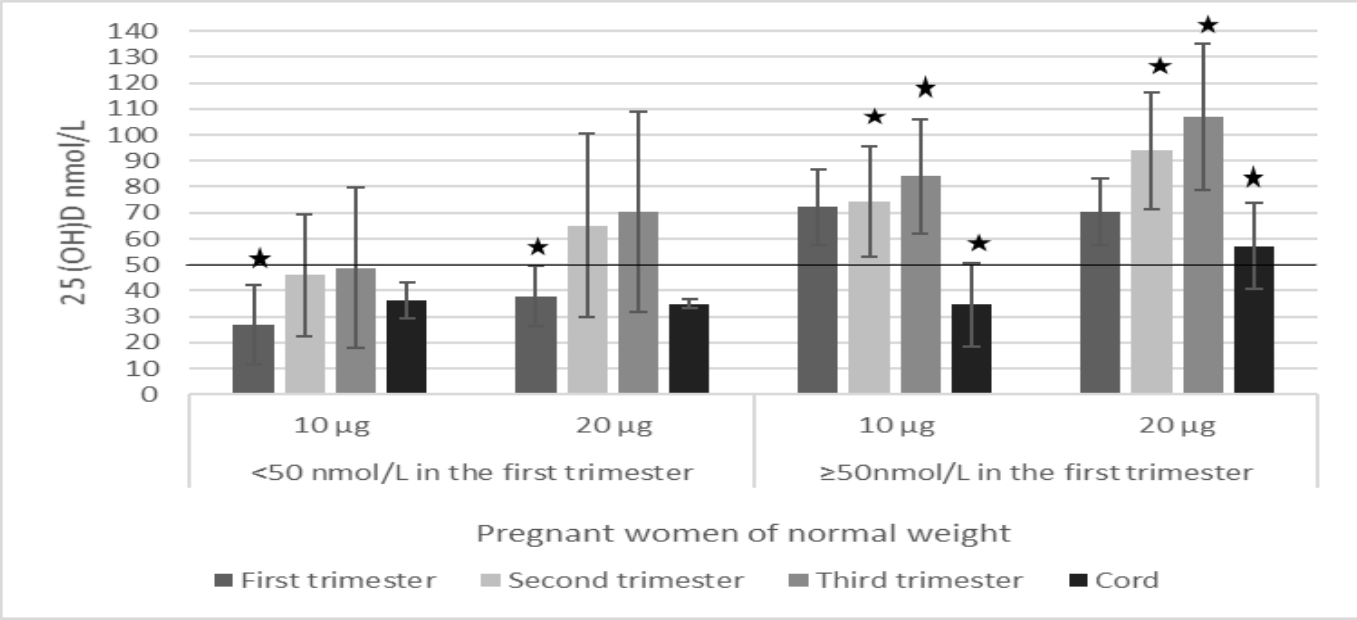

Figure 3 B. 25(OH)D concentrations in 10 µg and 20 µg groups in pregnant women of normal weight throughout pregnancy and in cord for those who started pregnancy with insufficient 25(OH)D concentrations <50 nmol/L and sufficient 25(OH)D concentrations ≥50 nmol/L. ★ Indicated to significant difference *P* (<0.05) between 10µg and 20µg groups who started pregnancy with insufficient (<50 nmol/L) and between 10µg and 20µg groups who started pregnancy with sufficient (≥50 nmol/L) were assessed by independent t-test.

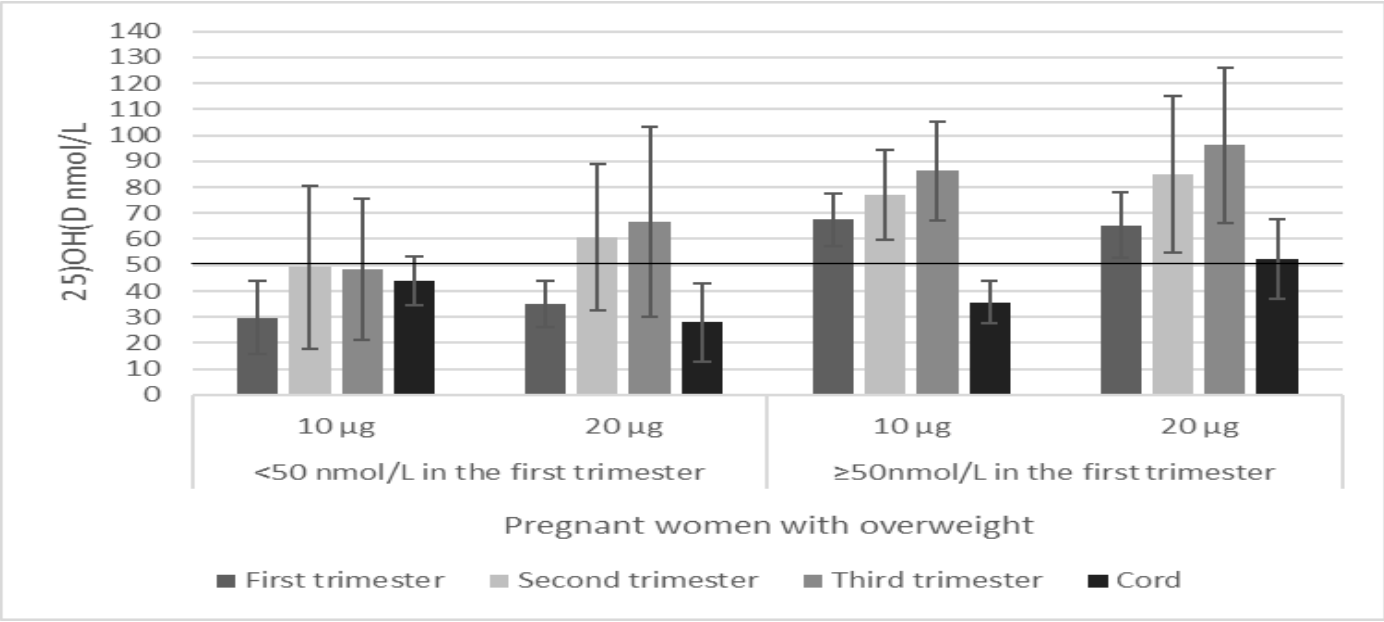

Figure 3 C. 25(OH)D concentrations in 10 µg and 20 µg groups in pregnant women with overweight throughout pregnancy and in cord for those who started pregnancy with insufficient 25(OH)D concentrations <50 nmol/L and sufficient 25(OH)D concentrations ≥50 nmol/L.

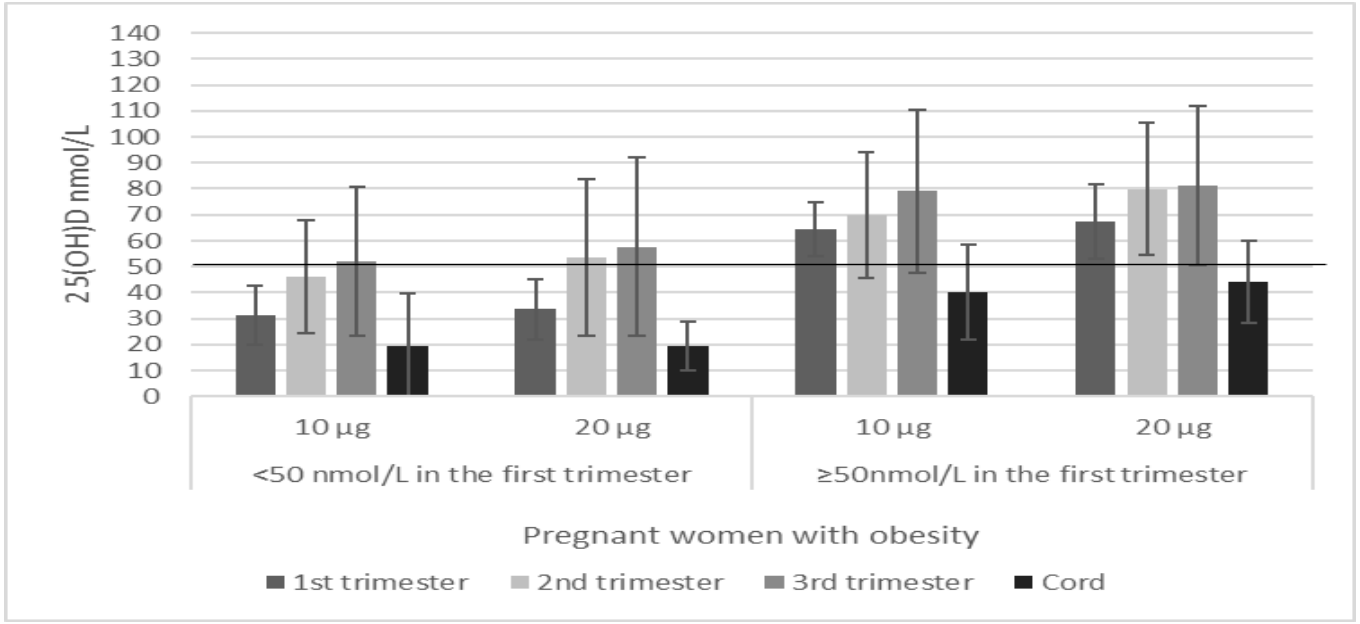

Figure 3 D. 25(OH)D concentrations in 10 µg and 20 µg groups in pregnant women with overweight throughout pregnancy and in cord for those who started pregnancy with insufficient 25(OH)D concentrations <50 nmol/L and sufficient 25(OH)D concentrations ≥50 nmol/L.

Chapter 6:

General Discussion

The overall aim of this thesis was to investigate the association between maternal body weight and vitamin D status throughout pregnancy. The prevalence of maternal obesity is increasing, with one in five women of reproductive age in the UK being classed as obese (Poston *et el.,* 2016; Devlieger *et al.,* 2016). The prevalence of vitamin D deficiency (<25 nmol/L) during pregnancy is largely unknown as the routine measurement of vitamin D is not part of standard antenatal care. A limited number of studies have reported on the prevalence of vitamin D deficiency to range from 13% to 64% (Yu *et al.,* 2009) and over 90% of pregnant women living in Northern Ireland have been reported to be vitamin D insufficient (<50 nmol/L), even with the use of vitamin D supplements (Holmes *et al.,* 2009). Previous studies have shown a negative association between maternal BMI and maternal vitamin D status (Perez-Lopez *et al.,* 2011; Bartoszewicz *et al.,* 2013). The complications of maternal obesity during pregnancy are similar to those reported as associated with vitamin D deficiency including increased risk of developing gestational diabetes mellitus (GDM), pre-eclampsia, having a preterm delivery and requiring a caesarean section. The adequacy of the current UK recommendation of 10µg/d (SACN, 2016) remains controversial, particularly in those who are overweight or obese, owing to the higher requirement and lower vitamin D status associated with obesity.

The main findings of this thesis are as follows: **Chapter 2**, the systematic review explored current knowledge on the association between maternal obesity and vitamin D status of mothers and their infants. In studies across the globe evidence shows that obese or severely obese pregnant women have consistently lower vitamin D status compared to non-obese pregnant women and maternal BMI is negatively associated with maternal vitamin D status. Infant vitamin D stores are directly related to maternal stores; studies report that vitamin D status is lower in infants born to obese mothers

compared to infants born to normal weight mothers. Maternal obesity was negatively associated with maternal and infant vitamin D status, with potential implications for both maternal and child health. Based on the systematic review it seems that public health advice on vitamin D supplementation during pregnancy should consider the implications of pre-pregnancy BMI. Findings from **Chapter 3 &4** further supported the existing literature regarding the association between body weight and vitamin D status during pregnancy**. Chapter 3** showed that in early pregnancy, women with obesity had significantly lower vitamin D status than those who were normal weight, this was evident particularly during the winter months. These data from the FASSTT trial collected in 2006, where women were not taking vitamin D supplements during pregnancy, showed that 60% of pregnant women living in Northern Ireland were considered vitamin D insufficient at 14 and 36 weeks gestation. **Chapter 4** showed that pregnant women classed as overweight and obese had significantly lower vitamin D status compared to pregnant women of normal weight in early pregnancy, despite 62% of women reporting to take a vitamin D containing supplement. Maternal BMI was found to be a significant negative predictor of vitamin D status. There was a high prevalence of vitamin D insufficiency with over 45% of pregnant women living in Northern Ireland considered insufficient, and this was highest among non-supplement users in winter months. The awareness of the importance of vitamin D supplementation may have increased over the time between the two cohorts in **Chapter 3 & 4**, which likely contributed to the increased vitamin D status from insufficiency in **Chapter 3** to sufficiency in **Chapter 4**. **Chapter 5** discussed the findings from a double-blind randomised vitamin D intervention study (MO-VITD) which assessed the effect of supplementation of 10µg vs. 20µg vitamin D_3/d throughout pregnancy on vitamin D status of normal weight, overweight and obese

pregnant women and on the cord blood of their infants. Maternal vitamin D status, increased from the 1st trimester to 3rd trimester in both the 10µg and 20µg groups, this increase was higher in the 20µg group. There was no overall difference in cord vitamin D status between the treatment groups. Vitamin D supplementation of 10µg/d is adequate to prevent pregnant women and their infant from vitamin D deficiency (<25 nmol/L) however is not enough to ensure maternal or cord sufficiency when women started pregnancy with insufficient vitamin D status. In this cohort 41.5% and 48.8% of pregnant women started pregnancy with insufficient status in the 10µg vs. 20µg treatment groups. Currently in the UK vitamin D status is not assessed during pregnancy, meaning these at-risk mothers remain unidentified. Recommendations advise all pregnant women to take 10µg/d of vitamin D however this is inadequate for those commencing pregnancy with the lowest vitamin D status. In the 20µg group, maternal and cord 25(OH)D concentrations reached and maintained sufficiency throughout the pregnancy, even in those who started pregnancy with an insufficient status. However, when taking pre-pregnancy BMI into account, this was not achieved across all BMI categories. In obese women who started pregnancy with an insufficient vitamin D status, the related cord blood vitamin D status was deficient in both the 10µg and 20µg groups. In contrast, obese women who started pregnancy as sufficient had cord blood results above deficiency and within the insufficiency range in both the treatment groups. In the 20µg group, pregnant women with obesity had lower vitamin D status in the second and third trimester and in cord compared to women of normal weight. Infants born to mothers with obesity who started pregnancy with insufficient vitamin D status are at higher risk of low vitamin D status (<25 nmol/L). This further highlights the need for vitamin D status to be measured as part of routine antenatal care, enabling HCPs to identify mothers most at risk of deficiency.

In **chapter 2** measures of BMI were reported to be taken either pre-pregnancy or throughout pregnancy. In contrast to this, in the studies included in **chapter 3, chapter 4 and chapter 5** all BMI measures were taken during early pregnancy at 12 and 14 weeks gestation with blood sampling for the analysis of vitamin D being carried out at the same appointment. This allowed for the accurate assessment of the association between body weight and vitamin D status and is one of the considerable strengths of this work.

Circulating 25(OH)D concentration is the most reliable biomarker for the measurement of vitamin D status, therefore it was used to establish vitamin D status in all blood samples in **chapter 3, chapter 4 and chapter 5**. The Institute of Medicine (IOM) has defined serum 25(OH) D concentrations greater than 50nmol/L as sufficient for bone health (Institute of Medicine, 2010). The cut-off point for deficiency has been defined as <25 nmol/L (SACN, 2016) or <30 nmol/L (IOM, 2011) and some researchers have argued that concentrations as high as >75 nmol/L are necessary for functions of vitamin D beyond bone health (Holick et al., 2011). However, pregnancy specific vitamin D guidelines outlining required intake are needed to prevent adverse outcomes. In this thesis the cut-off point for deficiency was defined as 25(OH)D <25 nmol/L, insufficiency 25-50 nmol/L and sufficiency ≥50 nmol/L, however further research in this area is urgently needed.

Seasonal variations are known to influence vitamin D status during pregnancy to account for this the studies were ongoing across all seasons. In **chapter 3** it was observed that during the winter months' obese mothers had significantly lower vitamin D status compared to their normal weight or overweight counterparts this difference was not observed during the summer months. In **Chapter 4** when examining baseline measures it was reported that vitamin D status in early pregnancy was significantly

lower in pregnant women with overweight and obesity compared to women of normal weight in winter months, but not in summer months. Vitamin D status of pregnant women with obesity who were also non-supplement users was significantly lower than supplement users with obesity, in winter months. In **chapter 5** when assessing the maternal percentage change of vitamin D, there was no difference in the baseline season in the group as a whole or among pregnant women in different BMI categories in both the 10µg and 20µg groups. These results demonstrate the additive effect that seasonal variability can have, particularly when coupled with a higher BMI and absence of supplement use compounding the risk for these women. Based on the geographical location in the northern latitude of the cohort studied in this thesis, vitamin D supplementation is required during pregnancy particularly during winter months and in those who have higher BMI to prevent vitamin D deficiency and implications related to maternal and infant outcomes. Season is a known contributor to having a lower vitamin D status, however this effect is further exacerbated in those with a higher BMI putting them at greater risk of vitamin D deficiency.

Season was the only significant predictor of vitamin D status in both early and late pregnancy **(chapter 3 & chapter 4)**, maternal BMI, supplement use and having been on a sun holiday were significant predictors of vitamin D status only in early pregnancy. However, in **chapter 5** season was not found to be a predictor of vitamin D percentage change during pregnancy.

Numerous different factors affect vitamin D status such as vitamin D supplement use, sun holidays and sun bed use; we able to account for the influence of these factors in **chapter 4** and in **chapter 5**, however in **chapter 3** only information on supplementation use was available.

Results reported in **chapters 4** and **chapter 5**, may have implications for policies on vitamin D supplementation during pregnancy as the current UK recommendation of 10µg/d, was not enough to prevent the high prevalence of vitamin D insufficiency found in early and late pregnancy. We also observed that the current recommendation of 10µg/d is not enough to ensure sufficient status in pregnant women with insufficient vitamin D status at early pregnancy; 10µg/d is adequate to prevent vitamin D deficiency but not enough to bring maternal and cord concentrations to sufficiency. Findings from this thesis would suggest that those planning a pregnancy should consider taking a vitamin D supplement in order to reach a sufficient status before conception. Due to the high prevalence of insufficiency in those who reported supplement use during early pregnancy, it may be that 20µg vitamin D is required to reach a sufficient pre-pregnancy status; further research is needed in this area.

In contrast, 20µg/d was able to increase maternal and cord vitamin D concentrations to sufficient status. The current study would suggest that a maternal vitamin D supplement of 20µg/d is needed to reach and maintain maternal and cord vitamin D status ≥50 nmol/L during pregnancy in all pregnant women living in Northern Ireland.

As we highlighted in this research, maternal BMI was negatively associated with vitamin D status during pregnancy, this was observed in non-supplement user and those taking 10µg/d supplements (**chapter 3** & **chapter 4**). Furthermore, among pregnant women taking 20µg/d in the second and third trimesters, obese pregnant women had lower vitamin D status than normal weight women. Moreover, we found that the cord vitamin D status was affected by maternal BMI; obese pregnant women had lower vitamin D cord status compared with the cord status of normal weight women in those who had taken 20µg/d supplements. Infants of obese women who enter pregnancy with a low vitamin D status had vitamin D deficiency even if the

mothers had taken 10 µg/d or 20µg/d, potentially putting infants at high risk for vitamin D deficiency and poor in-utero bone development. These findings are important for public health agencies reinforcing the need to consider maternal BMI when implementing recommendations for vitamin D supplementation.

The mechanisms behind lower maternal and cord vitamin D concentrations in women with higher BMI is not clear yet, it has been thought to be due to possible sequestration of vitamin D in adipose tissue. However further investigation is needed in this area to elucidate the mechanisms by which vitamin D is utilised *in vivo* during pregnancy particularly in women across BMI categories. In addition, the impact of maternal obesity and low vitamin D status during pregnancy on infants' bone health and outcomes is not yet fully investigated. Research in this area is required to identify an adequate vitamin D supplement dose, appropriate for use during pregnancy.

Key findings

- Pregnant women with obesity had lower vitamin D status than normal weight women in early and late pregnancy.
- Vitamin D deficiency was prevalent during winter in pregnant women and this was particularly evident in those with maternal obesity and non-supplement users.
- Maternal BMI was inversely correlated with maternal vitamin D status during pregnancy.
- Attaining sufficient vitamin D status in pregnancy through dietary supplementation is vital for the mother and child.

- 10µg/d of vitamin D supplementation during pregnancy is not enough to bring women who had an insufficient vitamin D status in early pregnancy to sufficient status throughout pregnancy.
- 20µg/d of vitamin D supplementation during pregnancy is required to increase maternal and cord vitamin D to sufficient status.

This thesis highlights areas for further investigations:

1. Investigation on the mechanisms of vitamin D metabolism during pregnancy on pregnant women with obesity compared to pregnant women of normal weight.
2. Further investigation of vitamin D transfer during pregnancy to the fetus from pregnant women with obesity compared to pregnant women of normal weight.
3. A need for studies to define thresholds for cord and infant vitamin D status that prevent adverse infant outcomes associated with low vitamin D status.

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Appendix 1:

Confirmation of Ethical Approval (Chapter 3)

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Research Office
Our Ref: NC:GOV

30 January 2014

Dr M McCann
Room W2036
School of Biomedical Sciences
University of Ulster
Coleraine

Dear Dr McCann

SPONSORSHIP FOR PROJECT REFERENCE 14/0002

Full Project Title: **The influence of overweight and obesity on vitamin D status during pregnancy**

Chief Investigator	Dr M McCann (Ulster)
Other investigators	Dr M Mulhern, Mrs R Alhomaid (both Ulster)

I confirm that the University of Ulster will act as sponsor for the above research project as required by the Research Governance Framework for Health and Social Care.

Please refer to the accompanying documentation for more information and to note the outline requirements placed upon investigators. In particular, you must seek appropriate ethical review and confirm that approval is in place before commencing the study.

Please do not hesitate to contact me should you require any further information.

Yours sincerely

Nick Curry
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Research Governance
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Research Office

UNIVERSITY OF ULSTER

RESEARCH GOVERNANCE

University Sponsorship of Research on Human Subjects conducted in collaboration with:

- **NHS trusts or other bodies and the HSC**
- **Other organisations requiring the University to act as equivalent to Sponsor**

Please find attached the University's letter confirming that it will act as Sponsor for this study.

The role of Sponsor carries the following requirements:

- Confirmation that arrangements are in place for the research to begin, including arrangements to manage and, where appropriate, fund the study
- Ensuring that the research protocol, the investigators and the environment are appropriate
- Confirming that ethical approval has been obtained before a study begins
- Seeking clinical trials authorisation (where appropriate)
- Ensuring that good practice arrangements are maintained for the duration of the study in relation to the conduct of the study, monitoring and reporting (including the immediate reporting of suspected unexpected serious adverse events or reactions)

In practice, the role of sponsor might be divided between the University and the NHS/HSC organisation hosting the research, but the requirements upon the investigators generally remain the same and these are: to conduct the study in line with the approved protocol, to maintain records, to provide reports as required during and at the end of the study and to report any adverse occurrences/seek approval for amendments to the protocol. You should also note that audits of compliance with procedures will be carried out by the NHS/HSC and the University from time to time.

In addition to complying with the University's requirements, you must also familiarise yourself with Trust (or equivalent organisation) policy in this area, including the requirement for honorary contracts to be in place.

Please do not hesitate to contact me should you require any further information.

Nick Curry
Senior Administrative Officer
Research Governance

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Research Office

UNIVERSITY OF ULSTER

RESEARCH GOVERNANCE

Project Reference Number 14/0002

Statement on indemnity for staff and students conducting research on human subjects

The University is indemnified, through its insurance policies (and subject to the terms and conditions of these policies), for its staff and students engaged in the pursuit of research involving human subjects where the research is being conducted for and on behalf, and with the prior knowledge and consent of, the University.

However, the University is not indemnified through its insurance for non-negligent harm. Legal liability does not arise where a person suffers harm but no-one has acted negligently. The University cannot offer advance indemnities or, generally, insure against non-negligent harm, although such indemnity can be applied for in specific cases and where it is considered to be an essential element of the study.

Participants in research studies (research subjects) should be made aware in the information provided to them of the University's position.

This statement is only valid if it is on headed paper, is signed and bears the Research Governance stamp.

This statement is current at 30 August 2012 but is subject to review by the University's insurers and legal advisors. Please ensure that the currency of this statement is confirmed before use.

NICK CURRY
SENIOR ADMINISTRATIVE OFFICER
RESEARCH GOVERNANCE

DATE: 24 February 2014

University of Ulster
RESEARCH
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Our Ref: NC:GOV

24 February 2014

Dr M McCann
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Lecturer in Human Nutrition
University of Ulster
Coleraine

Dear Dr McCann

Research Governance Reference Number: 14/0002
ORECNI Reference Number: 14/EE/0071
Title: The influence of overweight and obesity on vitamin D status during pregnancy

The Research Governance section has been advised that the above application has been given a favourable ethical opinion by an HSC/NHS ethics committee. Once you have gained permission from the Trust(s) involved, the research can proceed.

Please sign, date and return the attached Chief Investigator undertaking form and RETAIN all other documents for the study file, prior to commencing the research.

Please also note the additional documentation relating to research governance and indemnity matters, including the requirements placed upon you as Chief Investigator.

Further details of the University's policy are available at www.ulster.ac.uk/research/rg along with guidance notes, procedures, terms of reference and forms.

If you need any further information or clarification of any points, please do not hesitate to contact me.

Yours sincerely

Nick Curry
Senior Administrative Officer
Research Governance
028 9036 6629
n.curry@ulster.ac.uk

University of ULSTER

Research Office

Shore Road
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www.ulster.ac.uk

UNIVERSITY OF ULSTER

RESEARCH GOVERNANCE

Research on human subjects being conducted by staff and/or students of the University

Please find attached a letter from the University noting your application to undertake research involving human subjects/participants has gained a favourable ethical opinion from an HSC/NHS ethics committee and/or HSC Trust as appropriate.

The University's policy requires the Research Governance section to:

- Seek confirmation that arrangements are in place for the research to begin, including arrangements to manage the study
- Ensure that the research protocol, the investigators and the environment are appropriate
- Confirm that ethical approval has been obtained before a study begins, where required
- Ensure that good practice arrangements are maintained for the duration of the study in relation to the conduct of the study, monitoring and reporting (including the immediate reporting of adverse events)

The requirements upon the investigators are to:

- conduct the study in line with the approved protocol
- retain and maintain records, including hard copies of signed consent forms, appropriately
- provide reports as required during and at the end of the study
- report any adverse events
- seek prior approval for amendments to the protocol

In addition to complying with the University's requirements, you must also familiarise yourself with the requirements of any other organizations involved in the research as hosts or as funders.

Please do not hesitate to contact me should you require any further information.

Nick Curry
Senior Administrative Officer
Research Governance
028 9036 6629
n.curry@ulster.ac.uk

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Research Office

Research Governance

Undertaking by the Chief Investigator

To be returned following receipt of a favourable ethical opinion and/or HSC Trust permission and prior to commencement of the study

Name of CI: Dr M McCann
Project Ref: 14/0002
ORECNI Project Ref: 14/EE/0071
Project title: The influence of overweight and obesity on vitamin D status during pregnancy

Collaborating NHS/HSC organization: N/A

I understand that the University of Ulster has agreed to act as sponsor/co-sponsor or equivalent for the above project and that this places certain obligations upon me as Chief Investigator.

These are:

- to adhere to the research ethics, governance and other appropriate policies of the University and any NHS/HSC organization involved in the project
- to conduct the study in full compliance with the approved protocol
- to report any adverse events as required by the University and NHS/HSC procedures
- to provide interim and final reports on the progress and outcomes of the study
- to seek advance permission for any amendments or extensions to the project

I agree to the above and confirm that:

- the host NHS/HSC organization (where applicable) is aware of and supports this study
- a favourable ethical opinion has been obtained (where applicable) and the study will commence on

  date: 01-04-2014

  and end on

  date: 01-09-2014

Signed: Mary McCann Date: 06/03/2014
(Chief Investigator)

NHS
Health Research Authority
NRES Committee East of England - Norfolk
Nottingham REC Centre
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839368

14 February 2014

Dr Mary McCann
Lecturer in Human Nutrition
University of Ulster
W2036
University of Ulster, Coleraine
BT52 1SA

Dear Dr McCann

Study title:	**The influence of overweight and obesity on vitamin D status during pregnancy**
REC reference:	**14/EE/0071**
IRAS project ID:	**139030**

The Proportionate Review Sub-committee of the NRES Committee East of England - Norfolk reviewed the above application on 17 February 2014.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Ms Tracy Leavesley, NRESCommittee.EastofEngland-Norfolk@nhs.net

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved were:

Document	Version	Date
Evidence of insurance or indemnity	Ulster University	30 January 2014
Investigator CV	Dr Mary McCann	06 February 2014
Letter from Sponsor	Ulster University	30 January 2014
Other: CV	Dr Maria Mulhern	
Other: CV	Raghad Mohammed Alhomaid	
Protocol		
REC application	139030/561959/1/715	28 January 2014

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

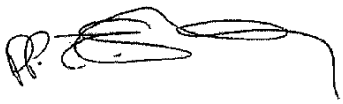
You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.
information is available at National Research Ethics Service website > After Review

14/EE/0071 **Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely



Dr Michael Sheldon
Chair

Email: NRESCommittee.EastofEngland-Norfolk@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Mr Nick Curry*

NRES Committee East of England - Norfolk

Attendance at PRS Sub-Committee of the REC meeting on 17 February 2014

Committee Members:

Name	*Profession*	*Present*	*Notes*
Mr Ron Driver	Retired Lecturer/Statistician	Yes	
Ms Leanne Groves	Psychological Therapist/Practice Development Facilitator	Yes	
Dr Elizabeth Lund (Alternate Vice-Chair)	Independent Consultant, Nutrition and Gastrointestinal Health	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Tracy Leavesley	REC Manager

Appendix 2:

Confirmation of Ethical Approval (Chapters 4-5)

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ulster.ac.uk

Our Ref: NC:GOV

11 June 2015

Dr M McCann
Room W2036
School of Biomedical Sciences
Ulster University
Coleraine Campus

Dear Dr McCann

Research Governance Reference Number: 15/0041
ORECNI Reference Number: 15/NI/0068
Study Title: Investigation of the impact of maternal obesity on vitamin D status during pregnancy: a randomised supplementation study

The Research Governance section has been advised that the above application has been given a favourable ethical opinion by an HSC/NHS ethics committee. Once you have gained permission from the Trust(s) involved, the research can proceed.

Please note the additional documentation relating to research governance and indemnity matters, including the requirements placed upon you as Chief Investigator.

1. **Please complete and return the Chief Investigator Statement of Compliance prior to commencing the study and keep a copy for your file.**

2. **Please retain all other documents.**

Further details of the University's policy along with guidance notes, procedures, terms of reference and forms are available at the following web address:

http://research.ulster.ac.uk/office/rofficeg.html

If you need any further information or clarification of any points, please do not hesitate to contact me.

Yours sincerely

Nick Curry
Senior Administrative Officer
Research Governance
028 9036 6629
n.curry@ulster.ac.uk

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ULSTER UNIVERSITY

RESEARCH GOVERNANCE

Project Reference Number: 15/0041

Project Title: Investigation of the impact of maternal obesity on vitamin D status during pregnancy: a randomised supplementation study

Statement on indemnity for staff and students conducting research on human participants

The University is indemnified, through its insurance policies (and subject to the terms and conditions of these policies), for its staff and students engaged in the pursuit of research involving human participants where the research is being conducted for and on behalf, and with the prior knowledge and consent of, the University.

However, the University is not indemnified through its insurance for non-negligent harm. Legal liability does not arise where a person suffers harm but no-one has acted negligently. The University cannot offer advance indemnities or, generally, insure against non-negligent harm, although such indemnity can be applied for in specific cases and where it is considered to be an essential element of the study.

Participants in research studies (research subjects) should be made aware in the information provided to them of the University's position.

This statement is only valid if it is on headed paper, is signed and bears the Research Governance stamp.

Nick Curry
Senior Administrative Officer
Research Governance

DATE: 11 June 2015

University of Ulster
RESEARCH GOVERNANCE

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ULSTER UNIVERSITY **RESEARCH GOVERNANCE**

Research on human participants being conducted by staff and/or students of the University

Please find attached a letter from the University noting that your application to undertake research involving human participants has gained a favourable ethical opinion from an HSC/NHS ethics committee and/or HSC Trust as appropriate.

Policies related to research in the HSC/NHS require the Research Governance section to:

- Seek confirmation that arrangements are in place for the research to begin, including arrangements to manage the study
- Ensure that the research protocol, the investigators and the environment are appropriate
- Confirm that ethical approval has been obtained before a study begins, where required
- Ensure that good practice arrangements are maintained for the duration of the study in relation to the conduct of the study, monitoring and reporting (including the immediate reporting of adverse events)

The requirements upon the investigators are to:

- conduct the study in line with the approved protocol
- retain and maintain records, including hard copies of signed consent forms, appropriately
- provide reports as required during and at the end of the study
- report any adverse events
- seek prior approval for amendments to the protocol

In addition to complying with the University's requirements, you must also familiarise yourself with the requirements of any other organisations involved in the research as collaborators, hosts or funders.

Please do not hesitate to contact me should you require any further information.

Nick Curry
Senior Administrative Officer
Research Governance
028 9036 6629
n.curry@ulster.ac.uk

1/7/15

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ULSTER UNIVERSITY **RESEARCH GOVERNANCE**

Chief Investigator Statement of Compliance

To be returned following receipt of a favourable ethical opinion and/or HSC Trust permission and prior to commencement of the study

Name of CI: Dr M McCann
Ulster Research Governance Study Ref: 15/0041
ORECNI Study Ref: 15/NI/0068
Study title: Investigation of the impact of maternal obesity on vitamin D status during pregnancy: a randomised supplementation study

Collaborating HSC/NHS organization:WHSCT

I understand that Ulster University has agreed to act as sponsor/co-sponsor or equivalent for the above study and that this places certain obligations upon me as Chief Investigator.

These are:

- to adhere to the research ethics, governance and other appropriate policies of the University and any HSC/NHS organisation involved in the study
- to conduct the study in full compliance with the approved protocol
- to report any adverse events as required by the University and HSC/NHS procedures
- to provide interim and final reports on the progress and outcomes of the study
- to seek advance permission for any amendments or extensions to the study
- where appropriate to register the study on a publicly accessible database

I agree to the above and confirm that:

- the host HSC/NHS organisation (where applicable) is aware of and supports this study
- a favourable ethical opinion has been obtained (where applicable) and the study will commence on

  date: 01-08-2015

  and end on

  date: 01-08-2017

Signed: Mary Mc Cann Date: 29-06-15
(Chief Investigator)

Business Services Organisation

Office for Research Ethics Committees Northern Ireland (ORECNI)

Customer Care & Performance Directorate
Office Suite 3
Lisburn Square House
Haslem's Lane
Lisburn
Co. Antrim BT28 1TW
Tel:+44 (0) 28 9260 3107
www.orecni.hscni.net
HSC REC A

11 June 2015

Dr Mary McCann
Lecturer in Human Nutrition
W2036, Ulster University
Cromore Road
Coleraine, BT52 1SA

Dear Dr McCann

Study title:	**Investigation of the impact of maternal obesity on vitamin D status during pregnancy: a randomised supplementation study.**
REC reference:	**15/NI/0068**
Protocol number:	**15/0041**
IRAS project ID:	**157618**

Thank you for your letter of 10 June 2015 , responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Kathryn Taylor, RECA@hscni.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **favourable ethical opinion** for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start

of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper		02 June 2015

Covering letter on headed paper		09 June 2015
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Indemnity Confirmation]		27 March 2015
GP/consultant information sheets or letters [Appendix 6 GP letter]	V1.0	15 January 2015
GP/consultant information sheets or letters [Appendix 7 GP letter abnormal result]	V1.0	23 February 2015
IRAS Checklist XML [Checklist_30032015]		30 March 2015
IRAS Checklist XML [Checklist_03062015]		03 June 2015
IRAS Checklist XML [Checklist_10062015]		10 June 2015
Non-validated questionnaire [Appendix 3 Health & Lifestyle Questionnaire]	2	02 June 2015
Other [CV C McKeown]		
Other [CV Dr M Parker]		
Other [Appendix 5 Data Collection Wk 12]	V1.0	15 January 2015
Other [Appendix 5 Data Collection Sheet]	V1.0	15 January 2015
Other [Appendix 5 Data Collection Delivery]	V1.0	15 January 2015
Other [Protocol for Foetal Death]	1	02 June 2015
Participant consent form [Appendix 2 Consent Form]	2	01 June 2015
Participant information sheet (PIS) [Appendix 1 Participant Information Sheet]	version 3	09 June 2015
REC Application Form [REC_Form_30032015]		30 March 2015
Referee's report or other scientific critique report [Scientific Peer Review]		09 February 2015
Referee's report or other scientific critique report [Appendix 11 Ethics Committee Review]	V1.0	20 February 2015
Research protocol or project proposal [Appendix 8 Research Protocol]	version 4	09 June 2015
Summary CV for Chief Investigator (CI) [CV Dr M McCann]		
Summary CV for student [CV R Alhomaid]		
Summary CV for supervisor (student research) [Dr M Mulhern]		
Validated questionnaire [Appendix 4 Food Frequency Questionnaire]		02 June 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

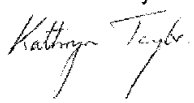
HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/NI/0068	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely



pp Dr Catherine Hack
Chair
Email: RECA@hscni.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Nick Curry, Ulster University*
Sally Doherty, Western Health and Social Care Trust

Western Health and Social Care Trust

3 July 2015

Dr Mary McCann
Lecturer in Human Nutrition
W2036 Ulster University
Cromore Road
Coleraine
BT52 1SA

Dear Dr McCann

Study Title: Investigation of the impact of maternal obesity on vitamin D status during pregnancy: a randomised supplementation study
HSC Trust Ref: WT 14/49 157618 (Please quote this number in all future correspondence)
REC Ref: 15/NI/0068

I am pleased to advise that WHSCT has given Final Research Governance Permission for the above project to commence. Permission is granted for the duration of the project to 01/06/17.

The following documents have been approved for use in the project:

Document	Version	Date
App 1 - PIS	3	09/06/2015
App 2 – Consent Form	2	01/06/2015
App 3 – Health Lifestyle Q	2	02/06/2015
App 4 – Final Version FFQ		02/06/2015
App 5 - Data Collection Sheet 12 weeks	1	15/01/2015
App 5 - Data Collection Sheet 28 weeks	1	15/01/2015
App 5 - Data Collection Sheet delivery	1	15/01/2015
Study process flow diagram		
App 6 - Letter to GP	1	15/01/2015

Research & Development Office, C-TRIC, Altnagelvin Area Hospital, Londonderry BT47 6SB
DDI 02871 611156
02871 345171 EXT 216603/4

Western Health and Social Care Trust

App 7 - Letter of abnormal result	1	23/02/2015
App 8 – Research Protocol	4	09/06/2015
App 9 – Protocol Occurrence of Miscarriage/Foetal Death	1	02/06/2015

The following personnel have been approved to work on the study at this Trust:

Name	Indemnity Provided by
Dr Michael Parker	WHSCT
Mrs Raghad Alhomaid	ULSTER
Miss Claire McKeaon	

Permission is granted subject to the attached conditions and I would ask you to please ensure that all members of the research team are familiar with these. Failure to abide by these conditions will invalidate permission and may result in the cessation of the research.

I wish you every success with your project.

Yours sincerely,

Dr Maurice O'Kane
Director of Research & Development

Cc Dr Michael Parker, Consultant Obstetrician and Gynaecologist

Research & Development Office, C-TRIC, Altnagelvin Area Hospital, Londonderry BT47 6SB
DDI 02871 611156
02871 345171 EXT 216603/4

Western Health and Social Care Trust

Conditions of Permission

Research Governance permission is issued provided the researcher(s) involved adhere to and abide by the conditions below.

- The researcher(s) must adhere strictly to the research protocol.
- There must be no changes to the research protocol or approved study documentation without the prior consent of the Trust, the Research Ethics Committee and, where applicable, the MHRA.
- There must be no changes in research staff without prior consent of the Trust.
- The Research Office should be informed if the Chief Investigator or Principal Investigator(CI/PI) is unable to continue to fulfil his/her duties as CI/PI for any reason such as long term absence, change in employment etc.
- There must be no increase in the resources required without prior consent of the Trust.
- Researcher(s) must report all untoward incidents and serious adverse events to the Trust.
- Any concerns in relation to the research protocol must be reported to the Trust.
- Researcher(s) must adhere to good research practice principles in line with the ICH Good Clinical Practice (GCP) guidelines.
- Researcher(s) must adhere to the Trust's Research & Development Standard Operating Procedures (available from the Research Office on request)
- On request, researcher(s) must make their research project available to Trust appointed monitors.
- The lead researcher must make an annual report to the Research Office for the duration of the project.
- The lead researcher should inform the Research Office on completion or termination of the project. Completion reports must be sent to the Research Office, Research Ethics Committee and, if applicable, MHRA.

Research & Development Office, C-TRIC, Altnagelvin Area Hospital, Londonderry BT47 6SB
DDI 02871 611156
02871 345171 EXT 216603/4

Appendix 3:

Research protocol (Chapters 4-5)

Impact of maternal body weight on vitamin D status during pregnancy

Background:

Vitamin D is involved in calcium and phosphate homeostasis and is essential for the maintenance of bone health. Severe vitamin D deficiency results in rickets in children and osteomalacia in adults (Bouvard *et al*., 2011; Christakos *et al*., 2011). Low vitamin D status has been implicated in the pathogenesis of a number of conditions including cardiovascular, autoimmune and inflammatory diseases and a number of cancers (Bouvard *et al*., 2011; Christakos *et al*., 2011). Vitamin D is a fat-soluble vitamin which is produced by the body after exposure to sunlight. Sunlight provides approximately 90% of vitamin D and the remaining 10% is provided by food such as fatty fish, egg yolks, liver, mushrooms and fish-liver oils (Holick *et al*., 2007; 2008). Many factors can limit vitamin D synthesis from sun exposure, such as latitude, season, sunscreen use, societal factors, pollution, weather, age, skin colour and body composition. One study has highlighted that the main cause of vitamin D deficiency in Ireland is its latitude which is between 51-55°N (Hill *et al*., 2004); this leads to reduced sunlight exposure for at least 6 months of the year during (autumn/winter) which time, vitamin D synthesis is negligible and individuals rely on stores (built up during the previous summer) as well as dietary sources.

The most robust biomarker for the measurement of vitamin D status is serum 25-hydroxyvitamin D (25(OH) D) concentrations, (Institute of Medicine, 2011) with concentrations greater than 50 nmol/L (or 20 ng/mL) defined as sufficient vitamin D status (Institute of Medicine, 2010).

Vitamin D deficiency (25(OH) D <30 nmol/L) and insufficiency (25(OH)D <50 nmol/L) is prevalent world-wide in many populations (Department of Health, 2011) with a higher prevalence in individuals considered at-risk (infants, elderly, pregnant women) (Bandeira 2006; Holick2007a). Indeed, many studies have reported vitamin D deficiency and insufficiency in pregnant women in many nations across the world (Dawodu *et al*., 2013). In Northern Ireland, it has been reported that 96% of pregnant women were vitamin D insufficient and insufficiency was prevalent even among those women who were supplement users (Holmes *et al*., 2009). Due to risk of bacterial exposure, food such as liver and eggs are advised to be avoided during pregnancy (Food Standards Agency, 2008), thus reducing the opportunity for the consumption of vitamin D rich food sources during this critical period of

development. Vitamin D is essential for maintaining normal bone health of the developing child (Bodnar *et al*., 2007) and mothers vitamin D status during pregnancy, which is directly correlated with offspring vitamin D status, has been shown to be a predictor of bone mineral density in the offspring later in life (Javaid *et al* 2006).

Studies have also highlighted that low maternal vitamin D status is a strong risk factor for conditions such as pre-eclampsia (Robinson *et al*., 2011; Bodnar *et al*., 2007), gestational diabetes (Wagner *et al*., 2012), hypertension (Wagner *et al*., 2012) and pre-term delivery (Dawodu *et al*., 2011). Furthermore, rickets, skeletal problems, type 1 diabetes, schizophrenia, and asthma are adverse health outcomes of infants born to mothers with a low vitamin D status during pregnancy (Holick 2006; McGrath 2001). Despite the high prevalence of vitamin D deficiency in pregnancy and its possible short and long-term consequences, the criteria for defining an optimal intake and status during pregnancy remains controversial (Holick *et al*., 2011; Wagner *et al*., 2012). In the UK, it is currently recommended that all pregnant and breastfeeding women should take a daily supplement of 10 µg of vitamin D (Food Standards Agency, 2008).

As previously mentioned, body composition is a determinant of vitamin D status. Observational and intervention studies have reported that those who are obese or overweight may need 2 to 5 times more vitamin D to reach a sufficient level when compared with lean individuals (Holick *et al*., 2011; Holick *et al*., 2007; Hossein-nezhad *et al*., 2013; Jorde *et al*., 2010;Lee *et al*., 2009). Vitamin D is fat-soluble and therefore may be sequestered in the larger pool of adipose tissue in overweight and obese individuals (Holick *et al*., 2011; Holick *et al*., 2007; Hossein-nezhad *et al*., 2013). In a study by Karani *et al*. (2013) it was shown that a higher BMI was associated with a lower vitamin D status in both men and women of various ages. Furthermore, another study has shown that cord blood 25(OH)D concentrations are significantly lower in infants born to obese mothers compared to infants born to lean mothers (Bodnar *et al*., 2007). This was further supported in 2013, in a study which demonstrated that at the same concentration of serum vitamin D, obese pregnant women transfer less 25(OH)D to the infant than normal-weight pregnant women(Jami *et al*., 2013) and the authors suggested that to transfer sufficient levels of vitamin D to the offspring, obese mothers may require a higher amount of vitamin D supplement than lean mothers, owing to reduced bioavailability and sequestration of 25(OH)D in adipose tissue (Jami *et al*., 2013).

We hypothesise that overweight and obese pregnant women have a lower vitamin D status than their leaner counterparts at the beginning of pregnancy and may need a higher daily supplementation level of vitamin D to achieve a sufficient status.

Therefore the primary aim of this randomised controlled intervention study is to assess the impact of 10 µg vs 20 µg/day of vitamin D on vitamin D status in normal weight, overweight and obese pregnant.

STUDY PROTOCOL

Methods

See (Figure 1) for a flow diagram of the study process.

Study Design

Double Blinded Randomised Controlled Intervention Study.

Study participants

240 pregnant women. Participants will meet the following inclusion / exclusion criteria:

Inclusion Criteria

- Pregnant women
- Age ≥18years
- BMI >18.5 kg/m²
- Without current pregnancy related complications
- At least 12 weeks gestation
- Have a singleton pregnancy (as confirmed at first scan)
- Pregnant women who are currently taking vitamin D and have had a sun holiday will be included in this study. All participants will agree to discontinue any current supplementation and will be provided with a multivitamin for the duration of pregnancy.

Exclusion Criteria

- Aged <18 years
- Pregnancy BMI <18.5kg/m^2
- Participants with multiple pregnancy
- Participants currently involved in another research study
- Participants with a history of gastrointestinal, hepatic, renal, vascular or haematological disorders.
- Participants who have had in vitro fertilisation (IVF) treatment
- Participants with a history of NTD affected pregnancies
- Pregnant women with active thyroid disease (e.g., Graves, Hashimoto or thyroiditis)
- Planned home births

Study Procedures

Recruitment:

At the booking appointment (approximately 9-10 weeks gestation), all pregnant women in the Western Trust Area will be given an information sheet with details of the study including contact details of the lead researcher should they wish to know more about the study. The midwife or the health care professional (HCP) will give a brief outline of the study and inform the potential participant that a researcher will be at the hospital clinic when they attend for their 12 week scan, to answer any further questions and to take consent, if they wish to participate in the research. The researcher will ensure that all participants meet the inclusion criteria and are fully aware of the requirements of the research. The total number of participants will be N=240, normal weight, overweight and obese pregnant women at least 12 weeks gestation. Using minim randomisation software and stratified by BMI, participants will be randomised by an independent person (clinical trials manager at Ulster University) to receive either 10µg or 20µg of vitamin D from 12 weeks gestation through to delivery. All participants will be given a pregnancy multivitamin which contains 10µg vitamin D. Participants will be randomised to receive a separate supplement of placebo (0µg) or 10µg vitamin D. Therefore all participants will receive 2 capsules to consume daily: a pregnancy multivitamin plus placebo (0µg) or a pregnancy multivitamin plus 10µg vitamin D. The placebo capsule and 10µg vitamin D will be matched for size, colour and texture.

Blood sampling:

The study will have 4 sampling time-points; 12 weeks gestation, 28 weeks gestation, 36 weeks gestation and a cord blood sample will be collected after delivery. At each time point a total of 20 ml (2x8ml serum tube and 1x4ml plasma tube) of blood will be collected. In addition, a 20 ml of cord blood sample will be obtained at delivery.

All blood samples will be taken by a fully trained phlebotomist and the cord blood sample will be collected by the midwife on duty at the delivery. All midwives have been trained in the collection of cord blood samples.

All blood samples will be analysed for vitamin D status, 25 hydroxyvitamin D (25(OH) D) and other markers of vitamin D metabolism will also be measured, such as 1, 25 di hydroxyl vitamin D (1, 25(OH)2 D), serum calcium and plasma parathyroid hormone concentrations (PTH). C - reactive protein (CRP), insulin, glucose, lipid parameters and bone turnover markers e.g. (serum alkaline phosphatase, bone specific alkaline phosphatase, osteocalcin, serum crosslops, tartrate-resistant acid phosphatase).

Also, blood samples will be analysed for pro- and anti-inflammatory cytokines and vascular markers e.g. (IL-6, IL-8, IL-10, TNF- α, IFN- γ, CRP, ICAM-1, VCAM-1).

DNA will be analysed by removing the buffy coat from blood samples to analyse genetic variants of interest in vitamin D metabolism e.g. (CYP2R1, CYP27B1, CYP24A1).

Measurements:

Bioelectrical impedance will be used for the measurement of body composition. At each time point (12, 28 and 36 weeks) maternal anthropometric and body composition measures (weight / height / BMI/ lean tissue / body fat/ TBW) will be taken and recorded. In addition, details from the projected growth chart for the foetus, routine blood and urine sample results during pregnancy will be taken from maternal notes.

Infants anthropometric measures at birth (weight / length/ head circumference) and other measures relevant to the health status of the mother and child will be recorded from maternal notes and paediatric charts.

All measurements will be carried out by trained researchers in a private environment. A data collection sheet will be used to record this information on which each subject will be identifiable only by an ID number.

Questionnaires:

Participants will be asked to complete a Health and Lifestyle Questionnaire at 12 weeks gestation and at 28 week gestations participants will be asked to complete a Food Frequency Questionnaire to assess vitamin D intake from foods.

A four day food diary will be used for the collection of dietary intake data. At the 28 week appointment, all participants will be given a food diary and detailed verbal and written instructions on how to complete the food diary. The food diary will be completed on 2 weekdays and 2 weekend days.

During each phase between appointments (12-28 weeks and 28-36weeks), participants will be contacted twice via telephone to evaluate how adherence to the study is being managed.

Data Analysis:

The Statistical analysis package for the social sciences (SPSS version 21) will be used to analyse all data collected during the study. Data will be examined for normality using the Kolmogorov-Smirnov test and where necessary, data may be log-transformed. Depending on the normality of data parametric or non-parametric equivalents will be used.

Primary outcome:

One Way ANCOVA will be used to assess the effects of 10 and 20µg/d vitamin D during pregnancy among lean, overweight and obese pregnant women using intention-to-treat analysis.

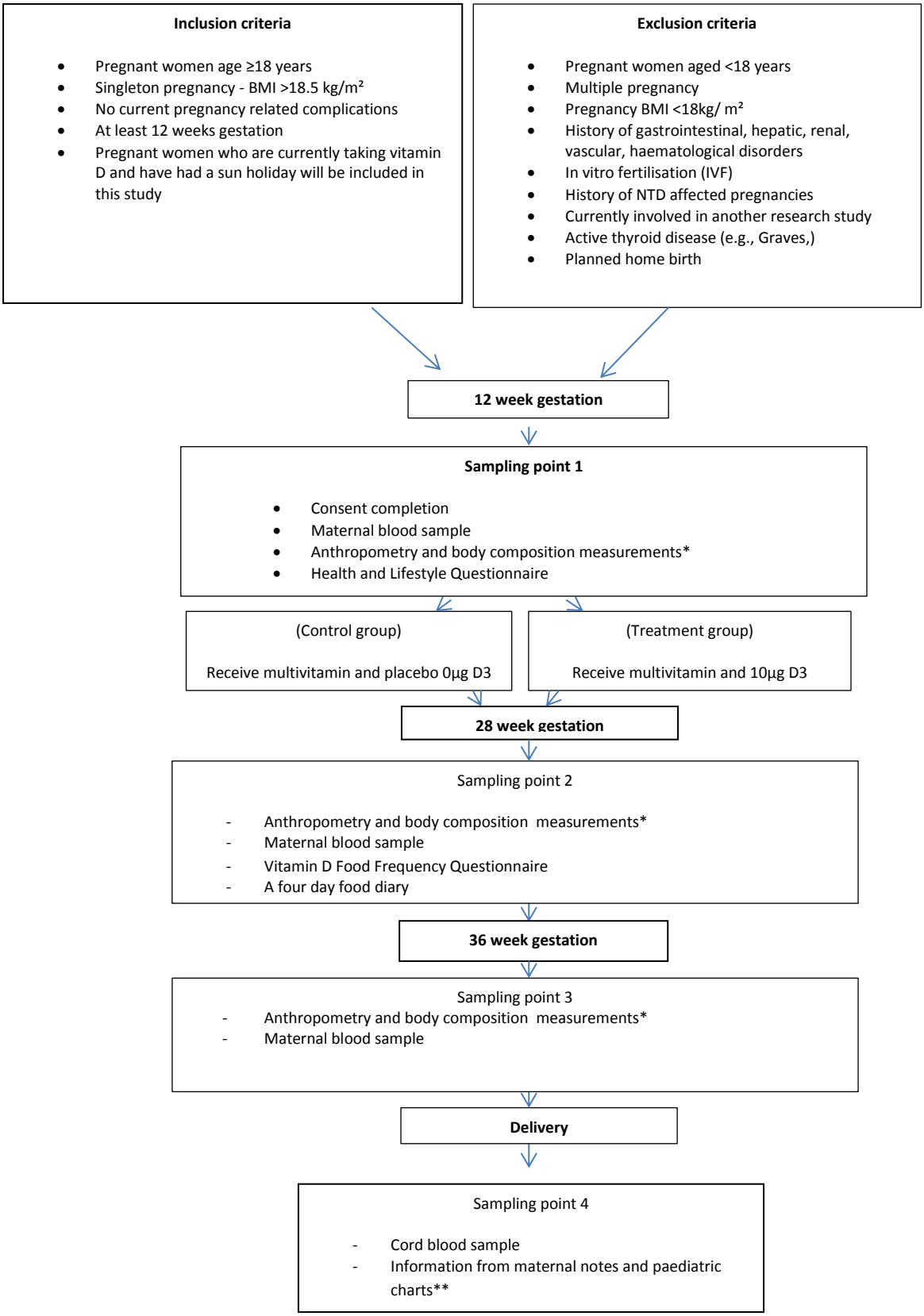
Secondary outcomes:

Regression analyses will be used to assess the relationships between vitamin D status and (BMI/ Age/ Infant anthropometric measurements/ mode of delivery/ inflammatory responses/ bone turnover markers and lipid parameters).P values <0.05 will be considered significant.

Confidentiality

All participants will be given a unique study identification code for identification purposes so that anonymity is protected at all times. All participant data will be stored on password protected computers. Hard copy personal data will be stored in locked cabinets under the custodial care of the principal investigator. Researchers will hold all information and data collected in confidence and all efforts will be made to ensure participants will not be identified (except as might be required by law or in disclosure of harm to self, neglect or illegal activity).

Figure 1: Obesity and vitamin D status during pregnancy intervention study



* Anthropometry and body composition measurements (weight/height/BMI/lean tissue/fat tissue/TBW)

** Duration of gestation, mode of delivery, birth weight/ Length /Head circumference, APGAR scores

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Appendix 4:

Participant Information Sheet (Chapters 4-5)

Appendix 1

Investigation of the impact of maternal body weight on vitamin D Status during pregnancy: a randomised supplementation study (MO-VITD Study)

Invitation

You are invited to take part in research being conducted at Ulster University in collaboration with your antenatal clinic at Altnagelvin Hospital. Before you decide whether or not to participate, it is important that you understand what the research is for and what you will be asked to do. Please read the following information and do not hesitate to ask any questions about anything that is unclear to you. Take time to consider whether or not you want to take part.

What is the purpose of the study?

There is currently a recommendation in the UK that all pregnant and breastfeeding women should be advised to take a supplement containing 10µg of vitamin D per day. It is well known that vitamin D is essential for bone health. Severe vitamin D deficiency can cause poor bone health in children and adults. Scientific research has shown that pregnant women living in Northern Ireland are at risk of vitamin D deficiency. Research studies have shown that a higher body weight is associated with a lower vitamin D status. Studies have also highlighted that pregnant woman with a higher body weight transfer less vitamin D to the infant than normal-weight pregnant women and it is thought that they may require 2 to 5 times more vitamin D during pregnancy than mothers with a lower body weight. We hypothesise that overweight and obese pregnant women have a lower vitamin D status than their leaner counterparts at the beginning of pregnancy and may need a higher daily supplementation level of vitamin D to achieve a sufficient level of vitamin D during pregnancy.

The main aim of this research study is to examine the adequacy of 10 vs. 20µg/day of vitamin D in normal weight, overweight and obese pregnant women in Northern Ireland and to determine if these supplementation doses are adequate to optimise vitamin D status.

Appendix 1

Why I have I been chosen?

You are being invited to take part as you are currently pregnant, and are considered otherwise healthy, with no current pregnancy related complications. All pregnant women within the Western Health and Social Care Trust are being invited to take part in this research study. In total, we will recruit approximately 240 pregnant women onto this research study.

Do I have to take part?

Whether or not you decide to take part, your routine care will not be affected in any way. If you do decide to take part, you will be given this information sheet to keep. If you choose to take part, you can change your mind at any time and withdraw from the study without giving a reason and without your clinical care or legal rights being affected. With your consent, the anonymised data or tissue already collected would be retained and used in the study.

What would taking part involve?

When you attend Altnagelvin hospital for your first scan, a researcher will be there to speak to you about the study and answer any questions you have. If you decide that you would like to take part in this research study, there are a number of criteria that we will check to ensure that you are eligible to take part. For example, you must meet the following criteria: age ≥18years; Body Mass Index (BMI) >18.5 kg/m²; without current pregnancy related complications; at least 12 weeks gestation; have a singleton pregnancy (this will be confirmed at your first scan).

Unfortunately you will **not** be eligible to take part if you meet any of the following criteria: aged <18 years; pregnancy BMI <18.5kg/m^2(underweight); pregnant with twins/triplets; currently taking part in another research study; have a history of gastrointestinal, hepatic, renal, vascular or haematological disorders; participants who have had in vitro fertilisation (IVF) treatment; pregnant women with active thyroid disease (e.g., Graves, Hashimoto or thyroiditis); have planned for a home birth delivery. Your previous obstetric history will be considered when determining eligibility for this research study.

Appendix 1

If you agree to take part in this study, you will be asked to sign a consent form. The researcher will ask you to complete a short questionnaire on your health and lifestyle; this will take no longer than 10 minutes. We will also take a small blood sample (20ml) and take measurements such as weight, height and body composition. We will measure your weight using a bioelectrical impedance analyser scale. This is just like stepping on a normal set of scales but this analyser also has the ability to calculate your percentage body water, muscle tissue and fat tissue. In total, your appointment with the researcher will take about 40 minutes.

All participants will be asked to take 2 tablets each day until the birth of their baby. This is a randomised control trial which means that participants receive one of the two treatments. The randomisation is completed by the clinical trials manager at Ulster University. This means that neither the researcher nor the research participant will know which treatment they are on.

You will receive either:

- a multivitamin tablet and an extra vitamin D tablet

  Or

- a multivitamin tablet and a placebo tablet

The placebo tablet is identical to the vitamin D tablet but does not contain the active vitamin D. It is a used as control measure to help understand the effects of the higher supplementation level of vitamin D. For example, we may expect that the participants taking the mutlivitamin and the placebo to have less of an increase in their vitamin blood levels than the participants taking the mutlivitamin and the extra vitamin D tablet. In this way we are able to compare the effectiveness of 10µg vs 20µg of vitamin D.

The multivitamin that you will receive is the *Pregnacare* multivitamin. So regardless of which treatment to which you are randomised, you will still receive the current recommended dose of 10 µg of vitamin D per day. We will ask that you discontinue any supplements that you are currently taking if you decide to take part in this research study.

All supplements will be supplied to you, free of charge from the date of your first scan until the delivery of your baby.

Ulster University

Appendix 1

Similar measurements will be taken when you attend the hospital for your 28 week appointment (blood sample and body composition measurements). In addition we will ask you to complete a short questionnaire about your normal intake of foods that contain vitamin D. Again this appointment will take approximately 40 minutes. We will give you a food diary to take with you and complete at a time most convenient to you. We ask that you record your food intake for 4 consecutive days. This will probably take about 10 minutes of your time each day that you complete the food diary.

When you have reached 36 weeks gestation, we will ask you to come to the research centre at Altnagelvin hospital for your final measurements. Again, we will take a blood sample and record your body composition measurements. This appointment will take no longer than 15 minutes. As this is not a routine appointment, any travel expenses incurred will be reimbursed.

After you delivery your baby, the midwife will take a sample of blood from the cord of the placenta. This gives us a measure of how much vitamin D you have been passing across to your baby during the pregnancy.

With your permission we would also like to access some relevant information from your maternal records (growth chart/ fundal height/routine clinic blood test/ urine results/method of delivery) and paediatric chart (baby weight/ baby length/ head circumference). We will also record some information on your general health and previous pregnancy history from your maternal notes. We will also check the routine blood test results that you will have had and record the results as a measure of your general health throughout the pregnancy. All blood samples will be taken by a fully trained phlebotomist and the cord blood sample will be collected by the midwife on duty at the delivery of your baby. The blood samples will be processed in the laboratory within 3 hours of collection and then stored at -80C° until analysis. All blood samples will be used to assess your response to the vitamin D supplementation. We will measure vitamin D status and other markers of vitamin D metabolism e.g calcium, and parathyroid hormone. In addition, DNA will be collected from blood samples to analyse genetic variants of interest in vitamin D metabolism. We would also ask for your permission to retain your blood samples for use in future studies.

Your GP will be informed that you are participant in the study and your consultant will be informed of any abnormal results, should they occur. The researcher will also keep in contact with you during the study and make telephone contact twice during each phase between your appointments (12-28 weeks and 28-36weeks).

Will my taking part in this study be kept confidential?

You will be given a unique study code for identification purposes so that anonymity is protected at all times. Your data will be stored on password protected computers. Hard copy personal data (such as consent forms, data collection forms) will be stored in locked cabinets under the custodial care of the principal investigator. Only the researchers involved in this study will have access to the anonymised information collected. Confidentiality will only be broken if it is believed that anyone is at risk of harm.

What are the possible benefits of taking part?

All participants involved in the study will receive a pregnancy multivitamin (Pregnacare brand) for the duration of their pregnancy. By taking a multivitamin tablet, it is highly likely that overall nutritional status during pregnancy will be improved.

What are the possible disadvantages and risks of taking part?

The study described above is highly unlikely to impact adversely on the health of the participants and all measures will be put in place to ensure this. There are no known risks of taking vitamin D 10µg supplements daily, as this is currently recommended to all pregnant and breastfeeding women in UK (Food Standards Agency, 2008). 20 µg of vitamin D daily is considered a safe level to consume during pregnancy.
Blood samples will be taken by a qualified phlebotomist who has undertaken a certified training course to ensuring any discomfort or chance of bruising will be kept to a minimum.

What if something goes wrong?

It is extremely unlikely that something will go wrong during this study. However, you should know that the University has procedures in place for reporting, investigating, recording and handling adverse events and complaints from study volunteers. The University is insured for its staff and students to carry out research involving people. The University knows about this

research project and has approved it. Further details on the complaints procedure can be found in the University's "Research Ethics and Governance" webpage http://research.ulster.ac.uk/rg/0208ResearchVolunteerComplaintsProcedure.pdf)

Any complaint should be made, in the first instance, to the chief investigator identified for this particular study (contact details at the end of this document). Any complaint you may make will be treated seriously and reported to the appropriate authority.

What will happen to the results of the study?

The results of this study will form part of a PhD project being undertaking by a student at Coleraine, Ulster University. The results may also be published in scientific journals. You will not be identified in any way in these publications. Depending on the findings of this research, the results may be used to improve advice or recommendations for vitamin D supplementation in pregnant women. We will send information on the main results of this study to all participants who took part.

Who has reviewed this study?

This study has been reviewed and approved by the Office for Research Ethics committee Northern Ireland (ORECNI), the Research and Development Office at the Western Health and Social Care Trust and the Biomedical Sciences Ethics Filter Committee at Ulster University.

Contact details

If you would like any further information, please do not hesitate to get in contact

Raghad Alhomaid, PhD student, Ulster University

028 (70) 123546 or email on: alhomaid-r@email.ulster.ac.uk

Dr Mary McCann, Chief Investigator, Ulster University

028 (70) 123969 or email on: mt.mccann@ulster.ac.uk

Thank you for your interest in this study

Appendix 5:

Consent Form (Chapters 4-5)

Appendix 2

Consent form for studies involving the use of human tissue

Participant Identification Number for this trial (ID):

Investigation of the impact of maternal body weight on vitamin D status during pregnancy: a randomised supplementation study (MO-VITD Study)

Chief Investigator; Dr Mary McCann

Additional Investigators; Dr Maria Mulhern, Mrs Raghad Alhomaid

Please confirm, by marking the boxes provided, that you agree with the following statements:

1	I have been given and have read and understood the information sheet for the above study and have asked and received answers to any questions raised.	☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my clinical care or legal rights being affected.	☐
3	I understand that the researchers will hold all information and data collected during the study securely and in confidence and that all efforts will be made to ensure that I cannot be identified as a participant (except as might be required by law or in disclosure of harm to self, neglect or illegal activity) and I give permission for the researchers to hold relevant personal data.	☐
4	I consent for my collected blood samples to be stored and used for the analysis in the current study and confirm that I have been given details of how it will be stored, used and the method of disposal.	☐
5	I consent for my DNA to be analysed as part of this study for the assessment of genetic variants of interest in vitamin D metabolism	☐
6	I consent for the researchers involved in this study to access the relevant sections of my maternal notes and paediatric charts to obtain information relevant to this research and to my data being used for the purpose of this research study as outlined in the information sheet.	☐
7	If I decide to withdraw from this study, I understand that no further data or tissue would be collected or any other research procedures carried out.	☐
8	If I decide to withdraw from this study, I consent that the anonymised data or tissue already collected would be retained and used in the study.	☐

9	I agree to be contacted by the research team regarding opportunities for future studies	☐

10 The potential benefits of keeping my blood or other tissues for future research studies have been explained to me and further research will only be conducted on my samples if I have provided enduring consent for this specific purpose:

a.	I consent to their indefinite storage and use in any future study, **or**	☐
b.	I consent to their indefinite storage and use in any future study that does not involve the isolation of my genetic material, **or**	☐
c.	I do not wish my blood or tissues to be used for any purpose other than this study	☐

Name of Participant (please print)	Signature	Date (dd/mm/yy)
Name of Researcher	*(Form to be on headed paper)*	

Appendix 6:

Health and lifestyle questionnaire (Chapters 4-5)

Appendix 3
ID: 327

Health and Lifestyle Questionnaire

Date seen: ☐☐ / ☐☐ / ☐☐
d d m m y y

Health and Social Care No: ☐☐☐

Personal Information:

Name (last) ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
(first) ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Date of brith ☐☐-☐☐-☐☐ Age: ☐☐

Address ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Telephone Number Home: ☐☐☐ - ☐☐☐ Work: ☐☐☐ - ☐☐☐

Mobile: ☐☐☐ - ☐☐☐ Email address: ☐☐☐☐☐☐☐☐☐☐☐☐

Socialdemographic Information:

1. Education level:

☐ Primary ☐ Secondary ☐ Diploma
☐ Degree ☐ Postgraduate ☐ Other……

2. Marital status:

☐ Single (never married) ☐ Married
☐ Separated ☐ Divorced ☐ Wido

Appendix 3
ID: 328

Health Information:

1. Do you take any medications on a regular basis? **Yes** ☐ **No** ☐

1a. If **yes,** please complete the table below:

Name	Dose please state number of pills or capsules or teaspoons consume	How often do you take these?			
		Daily Once/ twice/ three tim	Weekly	Monthly	Less often

2. Do you suffer from any medical illnesses? **Yes** ☐ **No** ☐

2a. If **yes**, what is the condition and when were you diagnosed?

_____

Supplement Information:

1. Do you take any vitamins, minerals, fish oils or other food supplements? **Yes** ☐ **No** ☐

1a. If **yes,** please complete the table below:

Name	Dose please state number of pills or capsules or teaspoons consume	How often do you take these?			
		Daily once/ twice/ three tin	Weekly	Monthly	Less often

1b. How long you have been taking these supplement?

_____

1c. What are the reasons for taken supplements?

☐ Preventive ☐ Therapeutic (prescribed by your doctor)

Appendix 3

ID: 329

Sun exposure Information:

1. Have you been on a sun or ski holiday in past 6 months? **Yes** ☐ **No** ☐

2. Have you been sunbathing or used a sunbed in the last months? **Yes** ☐ **No** ☐

   2a. If **yes**, have how many times per month did you use a sunbed ☐☐

   2b. If **yes**, have how long did each session last ☐☐ **miuntes**

Appendix 7:

Food frequency questionnaire (Chapter 5)

FOOD FREQUENCY QUESTIONNAIRE

VITAMIN D

Subject ID number: _____

Date: _____

THE FOLLOWING QUESTIONS ARE DESIGNED TO ESTIMATE THE AMOUNT OF VITAMIN D IN THE FOODS YOU USUALLY CONSUME. THEY ARE NOT TO CHECK IF YOU HAVE A HEALTHY DIET, OR TO CRITICISE WHAT YOU EAT AND DRINK. THANK YOU FOR TAKING THE TIME TO ANSWER THE FOLLOWING QUESTIONS CAREFULLY.

1. Do you drink milk?

Please tick the appropriate box.

YES	NO	NON-DAIRY

If yes, how often?

Please tick the appropriate box.

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Photograph G18 A	Photograph G18 B	Photograph G18 C
Whole (full)												
Semi-skimmed												
Skimmed												
Fortified												
Other: _____												

2. Do you usually have milk in your tea or coffee?

Please tick the appropriate box(es).

YES	NO

If yes, how often?

Please tick the appropriate box.

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Number of mugs	Number of cups
Tea											
Coffee											
Other:											

Which type of milk do you typically use in your tea or coffee (please tick)?

Whole (full) ☐
Semi – skimmed ☐
Skimmed ☐
Fortified ☐
Other ☐

3. Do you eat breakfast cereal?

Please tick the appropriate box.

YES	NO

If yes, how often and what kind of milk?

Please tick the appropriate box(es).

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Photograph G18 A	Photograph G18 B	Photograph
Whole (full)												
Semi-skimmed												
Skimmed												
Fortified												
Other: _____												

4. If you usually eat breakfast cereal what type of cereal do you eat and how much would you eat?

Please tick the appropriate box(es).

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ Day	2-3 times/ day	≥ 4 times/ day	Photograph 4	Photograph 5	Photograph 6	Photograph 7	Brand
Cornflakes														
Rice krispies														
All-bran														
Weetabix														
Shredded wheat														
Special K														
Porridge														
Other cereals Please name:														

5. Do you eat cereal bars?

Please tick the appropriate box.

YES	NO

If you usually eat cereal bars what type of cereal bars do you eat and how much would you eat?

Please tick the appropriate box(es).

Brand Please name:	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ Day	2-3 times/ day	≥ 4 times/ day

6. Do you usually eat milk pudding, rice pudding or custard?

If so, how often and how much would you eat each time?

YES	NO

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ Day	2-3 times/ day	≥ 4 times/ day	Photograph 14	Photograph 20
Milk pudding											
Rice pudding											
Custard											

7. Do you usually eat yogurt?

Please tick the appropriate box.

YES	NO

If yes, what type of yogurt and how often would you usually have it?

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Brand
Whole milk yogurt/low fat										
Fromage frais										
Drinking yogurt/yakult										
Other yogurt: Please name:										
Other drinking yogurt: Please name:										

How much yogurt would you usually eat?

	Number of Pots
Whole milk yogurt/low fat	
Fromage frais	
Drinking yogurt/yakult	
Other yogurt: Please name: _____ _____	

8. Do you usually eat butter/spread on your bread?

Please tick the appropriate box.

YES	NO

If yes, what type of butter/spread , how often would you usually have it and how much each time?

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Photograph 30
Butter										
Spreadable butter										
Olive-oil based spread										
Flora original/light/other polyunsaturated full/low fat spread										
Other spread: Please name: _____ _____										

9. Do you usually eat cheese?

Please tick the appropriate box.

YES	NO

If yes, what type of cheese do you usually eat, how often and how much would you eat?

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ Day	2-3 times/ day	≥ 4 times/ day	Photograph 24	Photograph 25	Photograph 26
Cheddar cheese												
Cheddar type cheese, low fat												
Processed cheese, plain												
Cheese spread, plain												
Cottage cheese												
Parmesan cheese												
Other cheeses: Please name: _____												

10. Do you usually eat bread?

Please tick the appropriate box.

YES	NO

	Brown	Whole Meal	White

Bread			
Rolls			
Wraps			

If yes, how often and what portion size each time?

Please tick the appropriate box(es).

	Bread	Rolls	Wraps
Seldom			
Once a month			
2-3 times/month			
1-2/week			
3-4/week			
5-6/week			
Once/day			
2-3 times/day			
≥ 4 times/day			
Photograph	G1	G2	

11. Do you eat bread from Subway?

YES	NO

Please tick the appropriate box.

If yes, please tick the appropriate box(es).

	Rolls
Seldom	
Once a month	
2-3 times/month	
1-2/week	
3-4/week	
5-6/week	
Once/day	
2-3 times/day	
≥ 4 times/day	

What size of Subway do you usually have?

6 inch ☐ 12 inch ☐

YES	NO

12. Do you usually eat cakes, chocolate and/or biscuits?

Please tick the appropriate box.

If yes, how often do you eat any of the following?

	Seldom	Once a month	2-3 times /month	1-2 times/week	3-4 times / week	5-6 times / week	Once a day	2-3 times / day	≥4 times / day	Number of bars / biscuits	Photograph 8	Photograph 9	Photograph 10	Photograph 11
Chocolate covered bar with fruit/nuts/wafer														
Milk chocolate														
Chocolates fancy fills (soft centres)														
Semi-sweet/shortbread biscuits														
Biscuits incl. Mikado, Kimberley														
Chocolate cake														
Sponge cake with/without cream														
Madeira cake/ Muffins														
Fruit cake														
Other cakes/biscuits Please name:														

13. Do you usually eat fish?

Please tick the appropriate box.

YES	NO

If yes, how often do you eat any of the following fish and what portion size each time?

Please tick the appropriate box(es).

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Photograph 42 (fish)	Photograph 43 (breaded fish)	Photograph G14 (can)	Photograph G10 (prawns)
Fish fingers/deep fried fish													
Plaice/Cod/Haddock													
Salmon/Trout													
Mackerel													
Tuna in water/oil													
Sardine in tomato/oil													
Other fish: Please name:													

14. Do you usually eat meat?

YES	NO

Please tick the appropriate box.

If yes, what type of meat do you usually eat and how often?

Please tick the appropriate box(es).

	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day
Sausages									
Pork									
Beef									
Beef dishes (lasagne, Curry, stew)									
Bacon									
Ham									
Lamb									
Black pudding									
Chicken									
Chicken dishes									
Other meat: Please name:									

If yes, how much do you usually eat?

Meat	**Photograph**	**Amount**
Sausages	G5	
Pork	32/G6	
Beef	32/33	
Beef dishes (lasagne, curry, stew)	34/37/38/39/72	
Bacon	G4	
Ham	G4	
Lamb	G6/32	
Liver	1 slice = 40g	
Black pudding	1 piece = 30g	
Chicken	41/ G9	
Chicken dishes	72	
Other meat: Please name:		

15. Do you usually eat eggs (e.g. cooked, in dishes e.g. omelette)?

Please tick the appropriate box.

YES	NO

If yes, how often?

Please tick the appropriate box(es).

	Scrambled	Fried	Boiled	Poached
Seldom				
Once a month				
2-3 times/month				
1-2/week				
3-4/week				
5-6/week				
Once/day				
2-3 times/day				
≥ 4 times/day				
Number of eggs used each time				

Please name the type of eggs eaten if different from hens eggs: _____

16. Do you usually consume drinks fortified with vitamin D?

Please tick the appropriate box.

YES	NO

If yes, how often do you consume these?

Name and brand of drinks fortified with vitamin D:	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day	Photograph G19	Photograph G17

17. Do you take any dietary supplements (vitamin/mineral tablets, cod liver oil, fish oil, herbal or other "natural" medicine?

Please tick the appropriate box.

YES	NO

Name of product	**Frequency (dose/ day)**	**Since when?**

Do you take this product on a regular basis throughout the year?

Please tick the appropriate box.

YES	NO

Name of supplement	Seldom	Once/ month	2-3 times/ month	1-2/ week	3-4/ week	5-6/ week	Once/ day	2-3 times/ day	≥ 4 times/ day

If not, please include details:

_____

Appendix 8:

Publications

List of publications

Published abstracts:

R. M. Alhomaid, M.S. Mulhern, E. Laird, J.J. Strain, M.B.E. Livingstone, H. McNulty, K. Pentieva, M.T. McCann (2015) Maternal obesity and associated vitamin D status in pregnancy: data from the FASSTT study. *Pediatria*, 5(12), 902.

R. M. Alhomaid, M.S. Mulhern, L. Cassidy, B. Mullan, A. McGuckin and M. T. McCann (2017) Estimated dietary vitamin D intake during pregnancy. *Proceedings of The Nutrition Society*, 76 (OCE3), E62.

R.M. Alhomaid, M.S. Mulhern, L. Cassidy, J.J. Strain, M.B.E. Livingstone, M. Healy, E. Laird and M.T. McCann (2018) The impact of maternal body weight on vitamin D status in early pregnancy. *Proceedings of The Nutrition Society*,

Maternal obesity and associated vitamin D status in pregnancy: data from the FASSTT study

R. M. Alhomaid, M.S. Mulhern, E. Laird, J.J. Strain, M.B.E. Livingstone, H. McNulty, K. Pentieva, M.T. McCann

Northern Ireland Centre for Food and Health (NICHE), Ulster University, Coleraine, BT52 1SA, UK

Introduction: Maternal obesity and vitamin D deficiency in pregnancy are both recognised as major public health issues. It has been reported that there is a twofold increase in vitamin D deficiency in pregnant women as maternal BMI increases from 22 to 34 kg/m^2.
Aim: To assess maternal vitamin D status (25-hydroxyvitamin D concentrations) among normal weight, overweight, obese.
Methods: Data and samples from a previous study (FASSTT Study) were used for analysis. Pregnant women without pregnancy complications, aged 18 to 35 years and having a singleton pregnancy were included. Participants were not taking any vitamin D containing supplements during pregnancy. Non-fasting blood samples were collected (at 14 and 36 weeks gestation) and were analysed for total serum 25(OH)D using liquid chromatography tandem mass spectrometry.
Results: A total of 216 pregnant women (135 normal weight, 57 overweight, 24 obese) were included in the current analysis. Obese pregnant women had significantly lower median vitamin D status when compared to normal weight women at 14 weeks gestation (32.15 vs.46.10 nmol/L; ***P***=0.038) but not at 36 weeks (34.65 vs. 42.10nmol/L; ***P***= 0.370). There was a significant negative correlation between maternal BMI and vitamin D status at 36 weeks gestation (**r**= -0.201; ***P***= 0.026). At 14 weeks gestation, 60% of participants were either deficient (25(OH)D <30nmol/L) or insufficient (25(OH)D<50nmol/L); with 50% and 20.8% of obese pregnant women classified as deficient at 14 weeks and 36 weeks gestation compared to 24.4% and 16.3% of normal weight women respectively.
Conclusion: Obese pregnant women are at high risk of vitamin D deficiency. These findings are important for public health agencies when setting recommendations for vitamin D supplementation during pregnancy.

Ulster University

Faculty of Life & Health Sciences

Maternal obesity and associated vitamin D status in pregnancy: data from the FASSTT study

R. M. Alhomaid, M.S. Mulhern, E. Laird, J.J. Strain, M.B.E. Livingstone, H. McNulty, K. Pentieva, M.T. McCann Northern Ireland Centre for Food and Health (NICHE)

Introduction

- Maternal obesity and vitamin D deficiency in pregnancy are both recognised as major public health issues.
- Obesity is considered one of the factors that can influence vitamin D status, owing to the fact that vitamin D is fat-soluble and thus may remain hidden within the adipose tissue[1,2,3].
- Higher BMI has been associated with a lower vitamin D status in different age groups of adults in both men and women[4].
- In pregnancy, there is a reported twofold increase in vitamin D deficiency in pregnant women as maternal BMI increases from 22 to 34 kg/m²[5].

Aim

To assess vitamin D status (25-hydroxyvitamin D concentrations) among normal weight, overweight, obese pregnant women.

Methods

- Data and samples from a previous study (FASSTT Study)[6] were used for the current analysis.
- Pregnant women without pregnancy complications, aged 18 - 35 years and having a singleton pregnancy were included.
- Participants were not taking any vitamin D containing supplements during pregnancy.
- At 14 weeks gestation weight and height were measured.
- Non-fasting blood samples were collected (at 14 and 36 weeks gestation) and were analysed for total serum 25(OH)D by measuring 25-hydroxyvitamin D2 (25(OH) D2) and 25-hydroxyvitamin D3 (25(OH) D3) using liquid chromatography tandem mass spectrometry.

Results

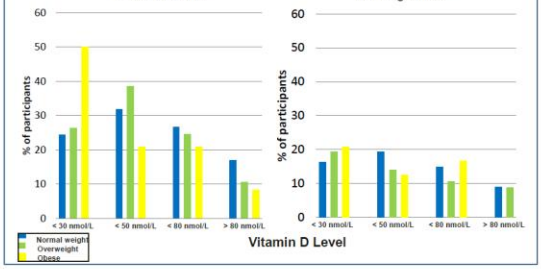
Table 1: Maternal vitamin D status across BMI categories

Vitamin D	N	Normal weight n(135)	Overweight n(57)	Obesity n(24)	ANOVA *P**
		Median (IQR)			
Vitamin D status at 14 week gestation	216	46.1 (30.3, 63.4)a	43 (28.6, 63.8)	32.1 (18.7, 61.3)a	0.038
		n(80)	n(30)	n(12)	
Vitamin D status at 36 week gestation	122	42.1(27.5, 65.9)	36 (20.1, 67.6)	34.6 (19.7, 55.6)	0.370
*P***		0.143	0.483	0.290	

Note: Data presented as median and interquartile range(IQR)
*P** <0.05 was considered significant *P*** Paired sample test

Figure 1a. Levels of deficiency / insufficiency and sufficiency at 14 weeks gestation

Figure 1b. Levels of deficiency / insufficiency and sufficiency at 36 weeks gestation



Conclusions

- Obese pregnant women had significantly lower vitamin D status when compared to normal weight women at 14 weeks gestation (32.1 nmol/L vs.46.1 nmol/L ***P***=0.038).
- There was a significant negative correlation between maternal BMI and vitamin D status at 36 weeks gestation (***r***= -0.201; ***P***=0.026).
- 50% and 20.8% of obese pregnant women were classified as deficient (25(OH)D <30nmol/L) at 14 and 36 weeks gestation.
- Obese pregnant women are at high risk of vitamin D deficiency.
- These findings are important for public health agencies when setting recommendations for vitamin D supplementation during pregnancy.

References

1. Holick, M.F. 2007, "Vitamin D deficiency", The New England journal of medicine, vol. 357, no. 3, pp. 266-281.
2. Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H., Weaver, C.M. & Endocrine Society 2011, "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline", The Journal of clinical endocrinology and metabolism, vol. 96, no. 7, pp. 1911-1930.
3. Hossein-nezhad, A. & Holick, M.F. 2013, "Vitamin D for health: a global perspective", Mayo Clinic proceedings, vol. 88, no. 7, pp.720-755.
4. Vimaleswaran K.S., Berry D.J., Lu C., Tikkanen E., Pilz S., et al. 2013,"Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts", ", PLoS medicine, vol. 10, no. 2, pp. e1001383.
5. Bodnar, L.M., Catov, J.M., Simhan, H.N., Holick, M.F., Powers, R.W. & Roberts, J.M. 2007, "Maternal vitamin D deficiency increases the risk of preeclampsia", The Journal of clinical endocrinology and metabolism, vol. 92, no. 9, pp. 3517-3522.
6. McNulty, B., McNulty, H., Marshall, B., Ward, M., Molloy, A.M., Scott, J.M., Dornan, J. & Pentieva, K. 2013, "Impact of continuing folic acid after the first trimester of pregnancy: findings of a randomized trial of Folic Acid Supplementation in the Second and Third Trimesters", The American Journal of Clinical Nutrition, vol. 98, no. 1, pp. 92-98.

Estimated dietary vitamin D intake during pregnancy. By R. M. Alhomaid, M.S. Mulhern, L. Cassidy, B. Mullan, A. McGuckin and M. T. McCann, *Northern Ireland Centre for Food and Health (NICHE), Ulster University, Coleraine, BT52 1SA, UK*

Vitamin D is involved in calcium and phosphate homeostasis and is essential for the maintenance of bone health[1]. Insufficient vitamin D intake has significant consequences for maternal and neonatal health. In Europe, maternal vitamin D intake has been reported to fall below the recommendations[2-3]. Higher BMI is associated with lower status of vitamin D but it is unclear if dietary intakes vary according to BMI, particularly during pregnancy. Some studies have reported higher intakes in obese pregnant women relative to normal weight women[4], whilst others have reported lower vitamin D intakes in obese compared to non-obese women [5].

The aim of this study was to assess and compare maternal dietary vitamin D intake among normal weight, overweight and obese pregnant women.

Data collected from an ongoing double-blinded randomised vitamin D intervention study (MO-VITD) were used for analysis. Pregnant women without pregnancy complications, aged >18 years and having a singleton pregnancy were recruited between January 2016 and December 2016. All participants completed a validated vitamin D food frequency questionnaire (FFQ)[6] at approximately 28 weeks gestation. Data from 80 pregnant women (43 normal weight, 20 overweight, 17 obese) were included in the current analysis.

The mean daily intake of vitamin D from food sources during pregnancy was 4.91 µg/d. Obese pregnant women had a significantly lower dietary vitamin D intake compared to normal weight women (3.19 vs. 5.57 µg/day; P=0.037). There was a significant negative correlation between maternal BMI and dietary vitamin D intake (r= -0.202; P= 0.036). When analysed at food level, reported vitamin D intake from fish, cereal, eggs and butter was 1.48, 1.33, 0.97 and 0.37 µg/d respectively. Breakfast cereals were the greatest contributor to vitamin D intake (27%) and only within the 'Fish' consumption group was there a significant difference in intakes across BMI categories, with obese pregnant women having a lower fish intake compared with normal weight women (1.95 vs. 0.66 µg/day; P=0.010).

The findings of this study are in agreement with other European research and demonstrate that maternal dietary vitamin D intakes are low. Maternal obesity is shown to be associated with dietary vitamin D intake. These findings support the guidelines for vitamin D supplementation during pregnancy.

1. Civitelli R, Ziambaras K (2011) *J Endocrinol Invest* **34**(7), 3-7.
2. Brembeck P, Winkvist A, Olausson H (2013) *Br J Nutr* **110**(5), 856-864.
3. Haggarty P, Campbell DM, Knox S *et al.,* (2013) *Br J Nutr* **109**(5), 898-905.
4. Karlsson T, Andersson L, Hussain A *et al.* (2015) *Clin Nutr* **34**(5), 892-898.
5. Scholl TO, Chen X (2009) *Early Hum Dev* **85**(4), 231-234.
6. Weir RR, Carson EL, Mulhern MS *et al.* (2016) *J Hum Nutr Diet* **29**(2), 255-261.

Estimated dietary vitamin D intake during pregnancy

Faculty of Life & Health Sciences

By R. M. Alhomaid, M.S. Mulhern, L. Cassidy, B. Mullan, A. McGuckin and M. T. McCann, *Nutrition Innovation Centre for Food and HEalth (NICHE), Ulster University, Coleraine, BT52 1SA, UK*

Introduction

- Vitamin D is involved in calcium and phosphate homeostasis and is essential for the maintenance of bone health[1].
- Insufficient vitamin D intake has significant consequences for maternal and neonatal health.
- In Europe, maternal vitamin D intake has been reported to fall below the recommendations[2-3]
- Higher BMI is associated with lower status of vitamin D but it is unclear if dietary intakes vary according to BMI, particularly during pregnancy.
- Some studies have reported higher intakes in obese pregnant women relative to normal weight women[4], whilst others have reported lower vitamin D intakes in obese compared to non-obese women [5]

Objectives

The aim of this study was to assess and compare maternal dietary vitamin D intake among normal weight, overweight and obese pregnant women.

Methods

- Data collected from an ongoing double-blinded randomised vitamin D intervention study (MO-VITD) were used for analysis.
- Pregnant women without pregnancy complications, aged >18 years and having a singleton pregnancy were recruited between January 2016 and December 2016.
- All participants completed a validated vitamin D food frequency questionnaire (FFQ)[6] at approximately 28 weeks gestation.
- Data from 80 pregnant women (43 normal weight, 20 overweight, 17 obese) were included in the current analysis.

Results

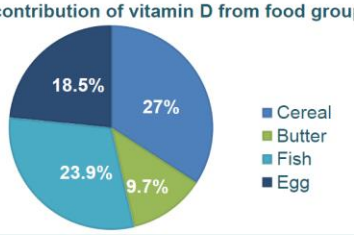
- Obese pregnant women had a significantly lower dietary vitamin D intake compared with normal weight women.

Maternal dietary vitamin D intake across BMI groups

Vitamin D intake(µg/d)	All n=80	Normal weight n=43	Overweight n=20	Obese n=17	P
Daily intake	4.91± 3.63	5.57 ± 4.13[a]	4.96 ± 3.12	3.19 ± 2.14[b]	0.037
Fish	1.48± 2.34	1.95 ± 2.74[a]	1.16 ± 1.51	0.66 ± 1.79[b]	0.010
Cereal	1.33± 1.48	1.17 ± 1.49	1.61 ± 1.59	1.43 ± 1.36	0.359
Eggs	0.97 ± 1.4	1.11 ± 1.62	1.08 ± 1.52	0.47 ± 0.55	0.155
Butter	0.37± 0.45	0.41 ± 0.56	0.35 ± 0.30	0.29 ± 0.27	0.862

*Data presented by mean ± SD and compared using Kruskal-Wallis Test.
Different superscript letters denote statistical difference.

Percentage contribution of vitamin D from food groups



Conclusions

- The findings of this study are in agreement with other European research and demonstrate that maternal dietary vitamin D intakes are low and this is most evident in obese women.
- Maternal obesity is shown to be associated with dietary vitamin D intake (r= -0.202; P= 0.036).
- These findings of low vitamin D dietary intake, support the guidelines for vitamin D supplementation during pregnancy.
- Further research is necessary to determine the influence of maternal obesity on vitamin d status during pregnancy.

References

1. Civitelli R, Ziambaras K (2011) *J Endocrinol Invest* **34**(7), 3-7.
2. Brembeck P, Winkvist A, Olausson H (2013) *Br J Nutr* **110**(5), 856-864.
3. Haggarty P, Campbell DM, Knox S *et al.*, (2013) *Br J Nutr* **109**(5), 898-905.
4. Karlsson T, Andersson L, Hussain A *et al.* (2015) *Clin Nutr* **34**(5), 892-898.
5. Scholl TO, Chen X (2009) *Early Hum Dev* **85**(4), 231-234.
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The impact of maternal body weight on vitamin D status in early pregnancy. By R.M. Alhomaid[1], M.S. Mulhern[1], L. Cassidy[1], J.J. Strain[1], M.B.E. Livingstone[1], M. Healy[2], E. Laird[3] and M.T. McCann[1], [1]*Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, BT52 1SA, UK,* [2]*Department of Biochemistry, Central Pathology Laboratory, St. James's Hospital, Dublin 8, Republic of Ireland and* [3]*School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Republic of Ireland.*

Maternal obesity and vitamin D deficiency are both public health issues for pregnant women. Vitamin D status (25-hydroxyvitamin D (25(OH)D) concentrations) during pregnancy has been shown to be inversely correlated with maternal body mass index (BMI)[1, 2]. In pregnant women, a 5 unit increase in BMI has been associated with a 25(OH)D lowering of 4.2 nmol/L and 2.8 nmol/L in winter and summer months, respectively[3].

The aim of this study was to assess and compare maternal vitamin D status between normal weight, overweight and obese pregnant women in early pregnancy.

Data collected at baseline from a double-blind randomised vitamin D intervention study (MO-VITD) were used for analysis. Pregnant women without pregnancy complications, aged >18 years and having a singleton pregnancy were recruited between January 2016 and August 2017 at antenatal clinics in the Western Health and Social Care Trust, Northern Ireland. Non-fasting blood samples were collected at 12 weeks gestation and were analysed for total serum 25(OH)D using liquid chromatography tandem mass spectrometry. Data from 239 pregnant women (80 normal weight, 79 overweight, 80 obese) were included in the current analysis.

The median (IQR) 25(OH)D concentration of all pregnant women at 12 weeks gestation was 52.9 (36.6, 67.2) nmol/L. Obese and overweight pregnant women were found to have significantly lower vitamin D status than normal weight women (49.6 (33.8, 64.0) vs. 51.8 (34.5, 62.8) vs. 58.8 (42.9, 74.5) nmol/L, P=0.014). A total of 44% of all pregnant women were found to be either vitamin D deficient (25(OH)D <25nmol/L;13%) or insufficient (25-50 nmol/L;31%) in early pregnancy. BMI was significantly negatively correlated with vitamin D status (r=-0.168; P=0.009). Season and supplement use were significant predictors of vitamin D status (β=0.153; P=0.013, β=-0.274; P<0.0001).

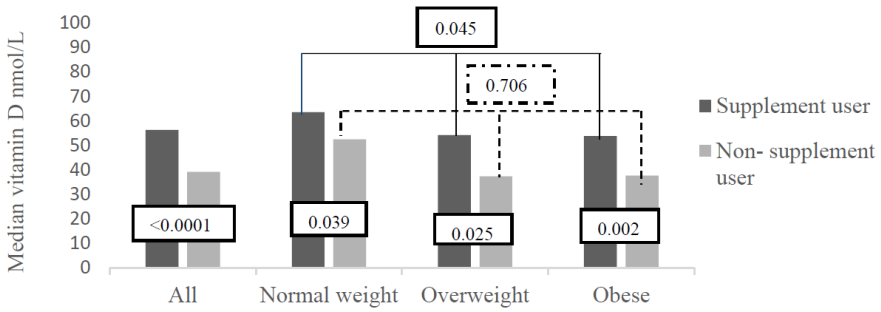

Figure 1. Vitamin D status of supplement users and non users acorss BMI groups

*P value for comparisons between supplement users and users using Mann-Whitney test and Kruskal-Wallis test between BMI groups

In early pregnancy, 62% of all pregnant women reported using a supplement containing vitamin D and 38% reported no supplement use. Supplement users had a significantly higher vitamin D status than non-supplement users in all BMI groups (**Fig. 1**).

In early pregnancy, obese pregnant women particularly non-supplement users, are at higher risk of vitamin D deficiency. These findings are important for public health services when setting recommendations for vitamin D supplementation during pregnancy.

1. Perez-Lopez, F. R., Fernandez-Alonso, A. M et al., (2011) *Reprod Sci*, **18**(8), 730-736.
2. Bartoszewicz, Z., Kondracka, A et al., (2013) *Ginekol Pol*, **84**(5), 363-367.
3. Andersen, L. B., Abrahamsen, B et al., (2013) *Clin Endocrinol, 79*(3), 333-341.

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The impact of maternal body weight on vitamin D status in early pregnancy.

By R.M. Alhomaid[1], M.S. Mulhern[1], L. Cassidy[1], J.J. Strain[1], M.B.E. Livingstone[1], M. Healy[2], E. Laird[3] and M.T. McCann[1], [1]Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, BT52 1SA, UK, [2]Department of Biochemistry, Central Pathology Laboratory, St. James's Hospital, Dublin 8, Republic of Ireland and [3]School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Republic of Ireland.

Introduction

- Maternal obesity and vitamin D deficiency are both public health issues for pregnant women.
- Vitamin D status (25-hydroxyvitamin D (25(OH)D) concentrations) during pregnancy has been shown to be inversely correlated with maternal body mass index (BMI) [1, 2].
- In pregnant women, a 5 unit increase in BMI has been associated with a 25(OH)D lowering of 4.2 nmol/L and 2.8 nmol/L in winter and summer months, respectively[3].

Aims

To assess and compare maternal vitamin D status between normal weight, overweight and obese pregnant women in early pregnancy.

Methods

- Data collected at baseline from a double-blind randomised vitamin D intervention study (MO-VITD) were used for analysis.
- Inclusion criteria: pregnant women without pregnancy complications, aged >18 years and having a singleton pregnancy.
- Pregnant women recruited between January 2016 and August 2017 at antenatal clinics in the Western Health and Social Care Trust, Northern Ireland.
- Non-fasting blood samples were collected at 12 weeks gestation and analysed for total serum 25(OH)D using liquid chromatography tandem mass spectrometry.
- Data from 239 pregnant women (80 normal weight, 79 overweight, 80 obese) were included in the current analysis.
- A Kruskal-wallis test was used to assess differences in maternal 25(OH)D concentrations between BMI categories.
- A Mann-Whitney test was used to assess differences in maternal 25(OH)D concentrations between supplement users and non-supplement users.
- Data presented as median (IQR) and $P<0.05$ considered significant.

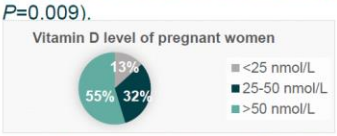
Results

- The median (IQR) 25(OH)D concentration of all pregnant women at 12 weeks gestation was 52.9 (36.6, 67.2) nmol/L.
- Obese and overweight pregnant women had significantly lower vitamin D status compared to normal weight women.

Table1. Maternal vitamin D status according to BMI category

	Normal weight n80	Overweight n79	Obese n80	*P*
25(OH)D nmol/L	58.8 (42.9, 74.5)[a]	51.8 (34.5, 62.8)[b]	49.6 (33.8, 64.0)[b]	0.014

- BMI of all pregnant women was 27.5 (23.8, 31.6) and was significantly negatively correlated with vitamin D status (r=-0.168; P=0.009).



- Season and supplement use were significant predictors of vitamin D status (β=0.153; P=0.013, β=-0.274; P<0.0001) respectively.
- 62% of all pregnant women reported using a supplement containing vitamin D.
- Supplement users had significantly higher vitamin D status than non-supplement users in all BMI categories.

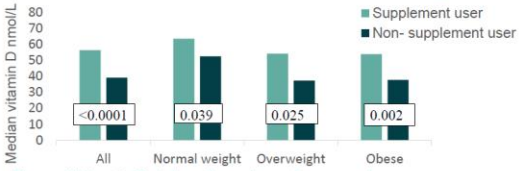


Figure 1.Vitamin D status of supplement users and non users acorss BMI groups

Conclusions

- In early pregnancy, obese pregnant women particularly non-supplement users, are at higher risk of vitamin D deficiency.
- These findings are important for public health services when setting recommendations for vitamin D supplementation during pregnancy.

References

1. Perez-Lopez, F. R., Fernandez-Alonso, A. M et al., (2011) Reprod Sci, 18(8), 730-736.
2. Bartoszewicz, Z., Kondracka, A et al., (2013) Ginekol Pol, 84(5), 363-367.
3. Andersen, L. B., Abrahamsen, B et al., (2013) Clin Endocrinol, 79(3), 333-341.

Appendix 9:

Presentations

Oral Communication

"Maternal obesity and associated vitamin D status in pregnancy: data from the FASSTT study" **Nutrition Society, Irish Section Postgraduate Meeting**, Radisson blue, Little Island, Cork, Ireland.